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GROWTH IN THE DENTAL ARCHES OF THE CAT[1]

A Daily Study from Birth through One Year of Development

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Previous studies on the dentition of the cat (1) (2) indicate that this animal, for a number of reasons, is especially suitable for studies related to human dentition. The life cycle of the cat's dentition follows closely that of the human. There are no teeth present at birth, and the deciduous teeth and permanent teeth both develop and erupt in the same relative order as those of the human dentition.

In this study, the relative growth values of the maxillary and mandibular dental arches, during their development from birth to the time of completion of the permanent dentition, were made from measurements of plaster models of the jaws of the cat. These measurements were compared with similar ones made directly on the jaws at intervals during the progress of the study, and demonstrated their accuracy (*fig. 1*).

TECHNIQUE

The animals were wrapped in a linen sheet and placed in a wooden box so constructed that only the head was exposed. As the kittens grew older anesthesia was used when necessary. Plaster impressions were taken of each jaw. Specially designed impression trays of copper were made at intervals during the growth period to approximate the sizes of the dental arches in both maxilla and mandible. An impression material that dissolves out in boiling water was used. The impressions were carefully varnished and cast in plaster of Paris, the plaster was permitted to set thoroughly, was then separated from the impression, and the model trimmed.

Two litter mates were used on alternate days over a period of a year. They were left with the mother until weaned, and then permitted to develop normally in roomy outdoor cages.

Models of the dental arches of both maxilla and mandible were measured anteroposteriorly from the distal surface of the last molar tooth to the medial of the first incisor tooth; and laterally, in the regions of the canine teeth, the premolar teeth, and the last molar teeth.

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[1] Read at a meeting of the New York Section of the International Association for Dental Research, June 2, 1938.

The measurements were made at 24 hour intervals from the day of birth to the 51st day of life, and thereafter each week until the 81st day. The final measurements were made one year after birth.

Table I (Mandible) and Table II (Maxilla) are compilations of the growth measurements described above, and include calculations of percentage growth based on maturity and birth dimensions.

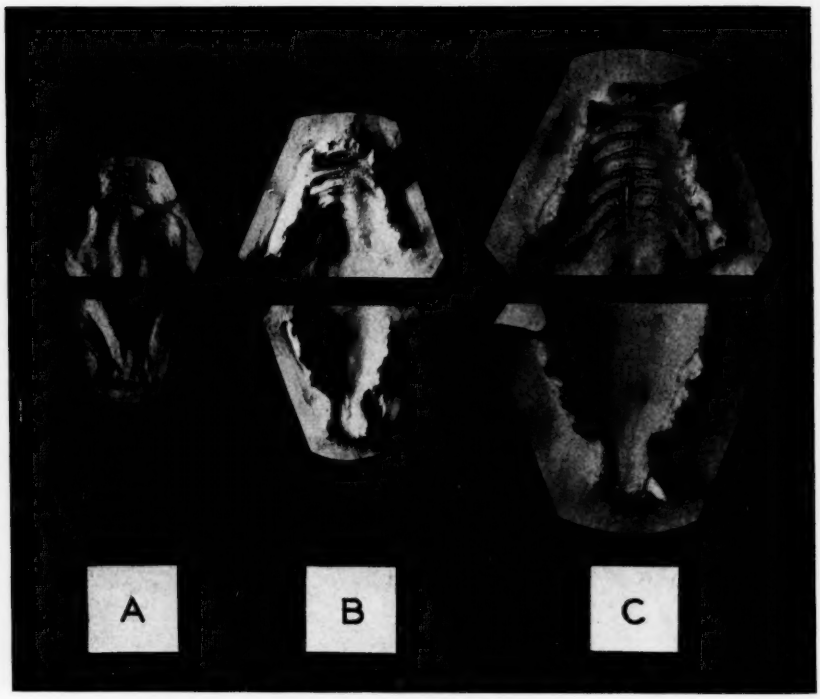

FIG. 1. Plaster models of maxilla and mandible of two litter mates used in the study at succeeding stages of growth. (a) Day of birth. (b) 81st day, when the deciduous dentition is complete. (c) 365th day, after the permanent dentition is complete.

OBSERVATIONS

Mandible

A–B, the anterior-posterior dimension represented in the graph by a solid line, has reached 36.54 per cent of its total percentage growth at birth, (*fig. 2*). On the 81st day it has grown to 75.27 per cent of

TABLE I.—*Mandible*

PERIOD IN DAYS AFTER BIRTH	MEASUREMENTS AT END OF PERIOD (DIMENSIONS IN M.M.)				TOTAL GROWTH GAINED AT END OF PERIOD (DIMENSIONS IN M.M.)				PER CENT OF MATURITY DIMENSION ATTAINED AT END OF PERIOD				PER CENT OF BIRTH DIMENSION GAINED AT END OF PERIOD			
	Average of A & B	C	D	E	Average of A & B	C	D	E	Average of A & B	C	D	E	Average of A & B	C	D	E
Birth	13.3	6.0	5.5	11.9	—	—	—	—	36.54	41.38	46.61	37.30	—	—	—	—
7th	16.0	7.2	7.0	15.5	2.7	1.2	1.5	3.6	43.96	49.64	59.32	48.59	20.30	20.0	27.27	30.25
14th	17.0	7.9	7.9	16.9	3.7	1.9	2.4	5.0	52.20	54.48	66.95	52.98	27.82	31.67	43.63	42.02
21st	20.4	8.7	8.0	17.6	7.1	2.7	2.5	5.7	56.04	60.00	67.80	55.17	53.46	45.00	45.45	48.90
28th	20.3	9.0	8.2	18.0	7.0	3.0	2.7	6.1	55.77	62.07	69.49	56.43	52.63	50.00	49.09	51.26
35th	20.9	9.0	8.5	19.0	7.6	3.0	3.0	7.1	57.42	62.07	72.03	59.56	57.14	50.00	54.54	59.66
42nd	21.2	9.6	8.1	18.9	7.9	3.6	2.6	7.0	58.24	62.21	68.64	59.25	59.40	60.00	47.27	58.82
49th	20.0	9.7	9.4	19.9	6.7	3.7	3.9	8.0	54.94	66.90	79.66	62.38	50.38	61.67	70.91	67.23
53rd	22.5	10.0	9.0	19.6	9.2	4.0	3.5	7.7	61.81	69.00	76.27	61.44	69.17	66.67	63.64	64.71
60th	23.7	10.8	9.8	20.3	10.4	4.8	4.3	8.4	65.11	74.48	83.05	63.64	78.20	80.00	78.18	70.59
67th	25.0	11.0	8.9	21.4	11.7	5.0	3.4	9.5	68.68	75.86	75.42	67.08	87.97	83.33	61.82	79.83
94th	27.3	11.2	9.3	22.4	14.0	6.2	3.8	10.5	75.00	77.24	78.81	70.22	105.3	103.3	69.09	88.24
81st	27.4	11.5	9.6	21.5	14.1	6.5	4.1	9.6	75.27	79.31	81.35	68.41	106.0	108.3	74.54	80.67
365th	36.4	14.5	11.8	31.9	23.1	9.5	6.3	20.0	100.0	100.0	100.0	100.0	135.3	158.3	114.5	168.1

TABLE II.—*Maxilla*

PERIOD IN DAYS AFTER BIRTH	MEASUREMENTS AT END OF PERIOD (DIMENSIONS IN M.M.)				TOTAL GROWTH GAINED AT END OF PERIOD (DIMENSIONS IN M.M.)				PER CENT OF MATURITY DIMENSION ATTAINED AT END OF PERIOD				PER CENT OF BIRTH DIMENSION GAINED AT END OF PERIOD			
	Average of A & B	C	D	E	Average of A & B	C	D	E	Average of A & B	C	D	E	Average of A & B	C	D	E
Birth	15.25	8.5	9.5	15.0	—	—	—	—	40.34	37.00	42.79	39.37	—	—	—	—
7th	15.80	9.5	11.0	15.0	0.55	1.0	1.5	0	41.80	41.30	49.55	39.37	3.55	11.77	15.79	0
14th	18.40	11.3	12.5	18.0	3.15	2.8	3.0	3.0	48.68	49.13	56.30	47.24	20.32	33.00	31.58	20.00
21st	19.80	12.0	12.5	20.2	4.55	3.5	3.0	5.2	52.38	52.17	56.30	53.02	29.35	41.17	31.58	34.67
28th	21.2	13.9	13.5	21.9	6.0	5.4	4.0	6.9	56.22	60.43	60.81	57.48	38.71	63.53	42.10	46.00
35th	22.05	13.3	14.1	22.4	6.80	4.8	4.6	7.4	58.33	57.83	63.51	58.80	43.87	56.50	48.42	49.33
42nd	23.20	14.3	15.5	22.3	7.95	5.8	6.0	7.3	61.37	62.17	69.82	58.53	51.29	68.23	63.16	48.70
49th	24.15	14.0	15.5	23.7	8.90	5.5	6.0	8.7	63.89	60.97	69.82	62.20	57.42	64.71	63.16	58.00
53rd	25.10	14.3	16.0	23.8	9.85	5.8	6.5	8.8	66.40	62.17	72.07	62.47	63.55	68.23	68.42	58.70
60th	27.0	14.5	16.6	24.6	11.75	6.0	7.1	9.6	71.43	63.04	74.77	64.57	75.81	70.60	74.74	64.00
67th	27.40	15.1	17.1	25.4	12.15	6.6	7.6	10.4	72.48	65.67	77.03	66.67	78.39	77.65	80.00	69.33
74th	27.80	15.2	17.6	25.6	12.55	6.7	8.1	10.6	73.55	66.09	79.28	67.19	80.97	78.82	85.26	70.70
81st	29.00	16.0	18.5	26.6	13.75	7.5	9.0	11.6	76.72	69.56	83.33	69.82	88.71	88.23	94.74	77.33
365th	37.80	23.0	22.2	38.1	22.55	14.5	12.7	23.1	100.0	100.0	100.0	100.0	145.5	170.6	133.7	154.00

its maximum, an increase of 38.73 per cent during the deciduous period. This leaves 24.73 per cent of the maximum to be attained during the period of permanent dentition. Based on birth dimensions, (*fig. 4*), A–B increases 106.0 per cent during the first 81 days and then grows to 135.3 per cent of its birth dimension during the period of permanent dentition.

C, the lateral canine dimension represented in the graph by an interrupted line, has reached 41.38 per cent of its maximum growth at birth, (*fig. 2*). On the 81st day it has grown to 75.27 per cent of its maximum, an increase of 38.73 per cent of its total percentage

TABLE III

	DIMENSION	PER CENT OF MATURITY DIMENSION (MATURITY = 100 PER CENT)				PER CENT OF BIRTH DIMENSION (BIRTH = 0 PER CENT)		
		At birth	At 81st day	Increase between birth and 81st day	Between 81st day and maturity	At 81st day	At maturity	Increase between 81st day and maturity
Mandible	A–B	36.54	75.27	38.73	24.73	106.0	135.3	29.3
	C	41.38	79.31	27.93	20.69	108.3	158.3	50.0
	D	46.61	81.35	34.64	28.65	74.54	114.5	39.96
	E	37.30	68.41	31.11	31.59	80.67	168.1	87.43
Maxilla	A–B	40.34	76.72	36.38	23.28	88.71	145.5	56.79
	C	37.00	69.56	32.56	30.44	88.23	170.6	82.37
	D	42.79	83.33	40.54	16.67	94.74	133.7	38.96
	E	39.37	69.82	30.45	30.18	77.33	154.00	76.67

growth. This leaves 20.69 per cent of the total percentage growth to be attained during the period of permanent dentition. Based on birth dimensions, (*fig. 4*) C increases 108.3 per cent during the deciduous period and then grows to 158.3 per cent of its birth dimension during the period of permanent dentition.

D, the lateral premolar dimension represented by a dotted line on the graph, reached 46.41 per cent of its total percentage growth at birth (*fig. 2*). On the 81st day it has grown to 81.35 per cent of its maximum, an increase of 34.64 per cent during the deciduous period. This leaves 28.65 per cent of the maximum to be attained during the period of permanent dentition. Based on birth dimensions, (*fig. 4*)

D increases 74.54 per cent during the deciduous period of 81 days and then grows to 114.5 per cent during the period of permanent dentition.

E, the lateral molar dimension represented on the graph by a dot-dash line, has reached 37.30 per cent of its total percentage growth at

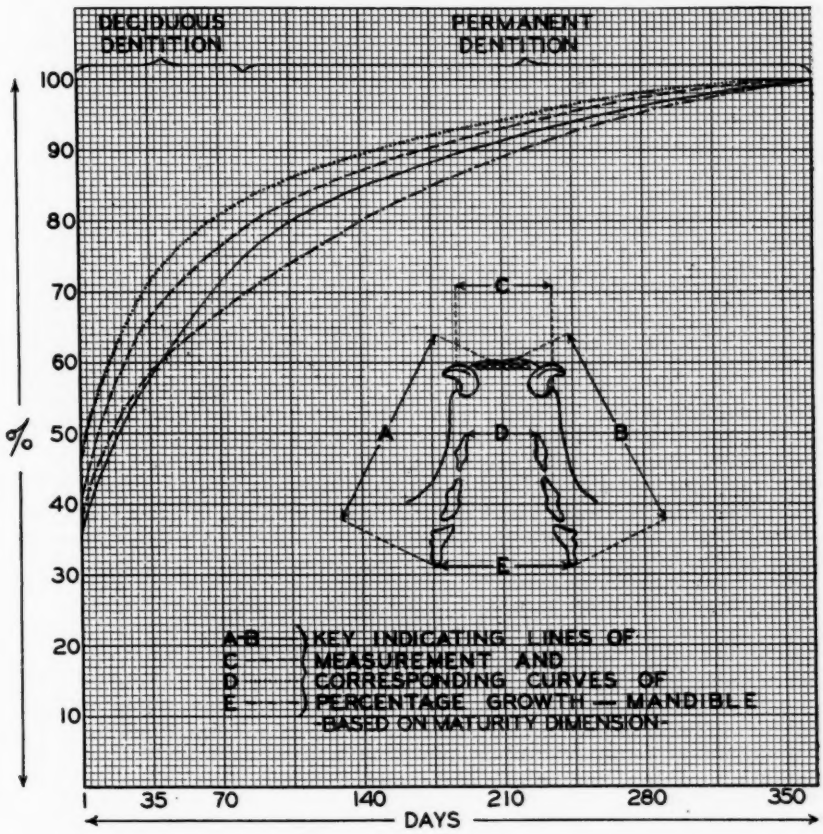

FIG. 2

birth (*fig. 2*). On the 81st day it has grown to 68.41 per cent of its maximum, an increase of 31.11 per cent during these 81 days. This leaves 31.59 per cent of the maximum to be attained during the period of permanent dentition. Based on birth dimensions, (*fig. 4*) E in-

creases 80.67 per cent during the first 81 days and then grows to 168.1 per cent of its birth dimension during the period of permanent dentition.

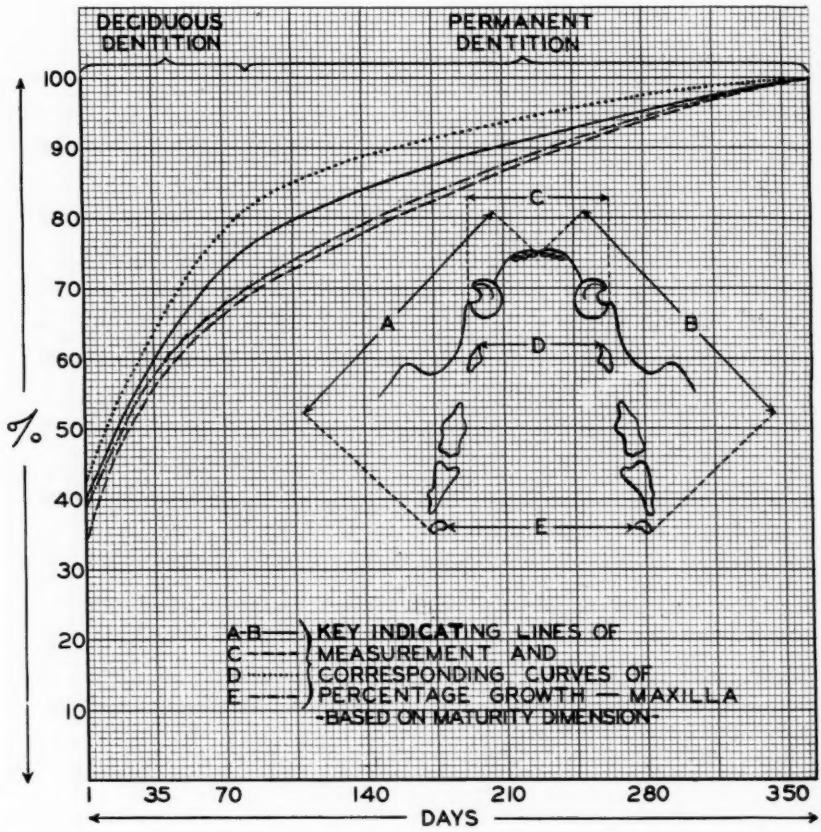

FIG. 3

Maxilla

A–B, the anterior-posterior dimension, has reached 40.34 per cent of its total percentage growth at birth (*fig. 3, solid line*). On the 81st day it has grown to 76.72 per cent of its maximum, an increase of 36.38 per cent of its maximum during these 81 days. This leaves

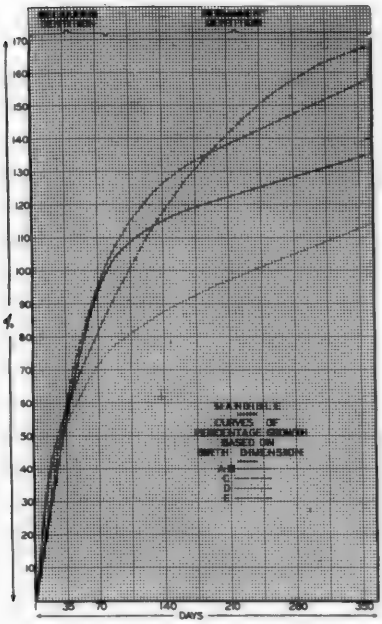

FIG. 4

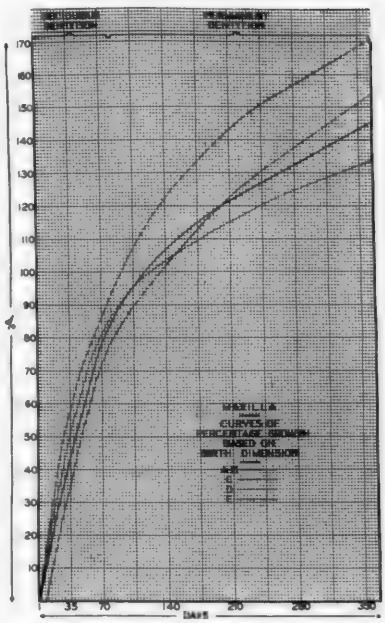

FIG. 5

EXPLANATION OF FIGURES 2–5

Figures 2, 3, 4 and 5 are curves of percentage growth in various dimensions of the mandible and maxilla as indicated in the outline diagrams. Ordinates indicate per cent of growth, abscissae age in days.

Figs. 2 and 3 are based on maturity dimensions, maximum growth being established as 100 per cent.

Figs. 4 and 5 are based on birth dimensions, size at birth being established as 0 per cent.

Lines A and B represent anterior-posterior dimension from median line immediately anterior to incisor teeth to distal gingival line of last molars. (Dimensions A and B are represented by a single curve as they are identical.)

Line C represents dimension across mandible in canine region using lateral gingival margins as points of measurement.

Line D represents dimension across the mandible in region of the first premolars using anterior gingival margins as points of measurement.

Line E represents dimension across mandible in region of last molar using distal gingival lines as measuring points.

The bracket marked "Deciduous Dentition" represents period of time from day of birth to 81st day of life when deciduous dentition is complete.

The bracket marked "Permanent Dentition" represents period from 81st to 365th day of life after permanent dentition is complete and jaws have attained maximum growth.

23.28 per cent of the total percentage to be attained during the period of permanent dentition. Based on birth dimensions (*fig. 5*) A–B increases 88.71 per cent during the 81 day deciduous period and then grows to 145.5 per cent of its birth dimension at maturity.

C, the lateral canine dimension, has reached 37 per cent of its total percentage growth at birth (*fig. 3, **interrupted line***). On the 81st day it has grown to 69.56 per cent of its maximum percentage growth, an increase of 32.56 per cent during the deciduous period, leaving 30.44 per cent to be attained during the remainder of the year. Based on birth dimensions, (*fig. 5*) C has increased 88.23 per cent of its birth dimension in 81 days and then to 170.6 per cent at the end of one year.

D, the lateral premolar dimension, has reached 42.79 per cent of its total percentage growth at birth (*fig. 3, **dotted line***). In 81 days it has grown to 83.33 per cent of its maximum, an increase of 40.54 per cent, leaving 16.67 per cent of the total percentage growth to be attained at the end of the year. Based on birth dimensions, (*fig. 5*) D has increased to 94.74 per cent of its birth dimension and reaches a maximum of 133.7 per cent during the period of permanent dentition.

E, the lateral posterior molar dimension, has attained 39.37 per cent of its total percentage growth at birth (*fig. 3, **dot-dash line***). By the 81st day it has grown to 69.82 per cent of its maximum, an increase of 30.45 per cent, leaving 30.18 per cent to be attained by maturity. Based on birth dimensions, (*fig. 5*) E has reached 77.33 per cent over its birth dimension and grows to a maximum of 154 per cent of the birth dimension.

Table III summarizes the observations described above.

CONCLUSIONS

A study of the findings indicates that the dimensional growth values vary during different periods of development.

Because the dentition of the cat follows the human curve so closely, and because it is accepted that the growth of the dental arches may be taken as an indication of the growth in the jaws, it would seem that this data may be used as a comparative basis for a similar study to be

made on humans. These findings may be of interest to orthodontists, who, in inducing growth in the arches, may work with rather than against the natural growth tendencies during certain periods of development.

The writer wishes to express appreciation for technical assistance rendered by Mr. Sidney Shapiro.

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DENTAL OBSERVATIONS IN GAUCHER'S DISEASE[1]

I. B. BENDER, D.D.S.

Philadelphia, Pa.

Dental roentgenograms have assumed increasing importance as an aid in diagnosis of general systemic diseases. Not only have they enabled the dentist to uncover dental pathologic conditions but also have aided in the recognition of primary lesions in the bones of the mandible and maxilla as well as the early manifestations of a generalized systemic disturbance. Not infrequently dental x-ray findings are an aid to the internist in establishing a tentative diagnosis. Paget's disease (1), Schüller-Christian's disease (2), parathyroid tumors (3), and coarctation of the aorta (4), are some of the diseases which have been detected by means of the dental intra-oral films. Since the dentist employs the x-ray almost routinely he is in the position to detect diseases of bone of systemic origin. Schroff (5) in taking routine roentgenograms of the teeth discovered the first evidence of Schuller-Christian's disease in the mandible of a patient who was otherwise asymptomatic. Here for the first time, intra-oral x-ray signs of Gaucher's disease are described.

Gaucher's disease was first described as "Epithelioma de la Rate" by Gaucher (6) in 1882. This disease is one of a group usually described under the general heading of xanthomatoses. Belonging to this group are the following: Gaucher's disease, Neiman-Pick disease, Schüller-Christian's disease, Tay-Sach's disease and xanthomatoses of skin. That each one of these diseases is due to a disturbance of lipoid or fat metabolism has been shown by Epstein and Lorenz (7). Leib (8) identified the lipoid at fault in Gaucher's disease, one of the cerebrosids, and called it kerasin. According to Pick (9), kerasin is stored in foam cells of the reticulo-endothelial

[1] From the Dental and X-Ray Departments of the Jewish Hospital. Delivered before the Academy of Stomatology, February 18, 1938; awarded honorable mention for the Benjamin Lord Prize, First District Dental Society of New York, 1937.

system, especially in those of the spleen and liver. These foam cells usually measure from 20 to 100 μ in diameter and are known as Gaucher cells. In Gaucher's disease the foam cells invade the spleen, liver, lymph nodes and bone marrow. Pick (9) is of the opinion that the Gaucher cells are never found in the blood stream, cortex of bone, or in the periostium, although di Guglielmo (10) did find Gaucher cells in the blood stream. This author reports a case for the first time with Gaucher cells in the bone cortex and the periostium.

Gaucher's disease is usually predominant in the Jewish race. It appears to be more frequent in the females. Clinically it is characterized by a slow chronic course with a gradual increase in the size of the spleen followed by an enlargement of the liver, the superficial lymph nodes remaining normal in size. The patient develops certain blood changes: First, there is a leukopenia then a hypochromic anemia. A thrombocytopenia is present which is related to the hemorrhagic diathesis characterizing the late stages of the disease. This condition may be due to the infiltration of the bone marrow by foam cells. Most often there is bleeding from the gums and nose. Involvement of the bone is usually characterized by pains in the femur and tibia or by tenderness over the sternum and ribs. In some patients the pain in the bones may be so severe as to mistake it for an acute osteomyelitis, as was done in case 1. This pain may be attributed to the increased subcortical pressure caused by the infiltrating masses of Gaucher's cells. An inconstant symptom, described by many authors, is a yellow-wedge shaped pinguecula of the sclera and conjunctiva. Hoffman and Mackler (11) summarized 89 cases and found this sign present in only 14 instances, appearing even less frequently in children. The bleeding time and coagulation time are usually within normal limits providing there are no complicating hemorrhagic phenomena present.

The skeletal changes in Gaucher's disease as pointed out by Pick (9) have elicited interest following the radiographic observations of Junghagen (12) and Fisher (13). According to Pick, the bones appear less opaque because of the generalized atrophy and calcium deficiency; coarse grained and worm eaten because of the extreme porosity. The cancellous bone may show large defects together with thinning of the cortex. Collapse and deformity of the head of the

femur are visible in the x-ray; club like appearance of the lower femur is often evident. There is apparently a reduplicated cortex, the intervertebral discs appear unchanged. There are no reactive or regenerative processes in the bone and periosteum and the cartilage is always intact.

In some instances the bone lesions have been a predominant manifestation of the disease which has led to the diagnosis. These according to Junghagen are: 1—Generalized osseous decalcification, 2—porosity of the trabecular structure, 3—destruction of the spongy bed with a worm eaten appearance of the tissue, 4—periosteum and cartilage are intact. Fisher added other radiographic signs: 1—Thinning of the cortex, 2—enlargement of the inferior extremity of the femoral bone, dilated into a bottle-like form, 3—compression of the head of the femur and the vertebra.

The following 2 case reports are similar examples of osseous dysplasia with manifestations in the bones of the jaw. The first case, a typical example of Gaucher's disease was reported from the medical standpoint by Capper, Epstein and Schless (14). The second case is unusual because lesions were found in the cortex of the bone and despite this the dental findings strongly suggested the diagnosis of Gaucher's disease. The second case will be published by Levine (15) and Solis-Cohen at a later date.

Case 1: A Jewish girl, age 13, presented herself in May, 1935 to have 2 lower first molars extracted. Examination of the mouth revealed that the lower first molars were badly broken down from the mesial aspect, there was pulpal involvement in the gangrenous state, the mucous membranes appeared normal, there were no areas of inflammation, abrasion or petechial spots, the gingivae were normal, having no pockets, extravasations or exudation on pressure, the floor of the mouth, the hard and soft palate were essentially negative. Except for the lower molars the electric pulp tester revealed normal responses in all teeth. The lower first molars were extracted under nitrous oxide and oxygen anesthesia. The patient was instructed to return 2 days later for routine x-ray examination, and examination then revealed no periapical infection. The lower second molars showed areas of incomplete calcification, the third molars were found to be unerupted, the first molar sockets were normal and showed areas of marked radioluscence especially in the molar and premolar zones (*fig. 1*). These areas presented a definite multiple cyst formation, and as is often seen in a true cyst, the wall was not well defined. The teeth remained fixed in their position, the lamina dura and peridental membrane did not appear to be destroyed by the supposed existent pressure. The nest of cells or cysts seems to have a foamy appearance and a few strands of bone structure were noted to be present within the cells. The mental region of the mandible presented a generalized porosity to a slight degree. The maxillae had a demineralized appearance with a particular area of lysis in

the upper left premolar region (*fig. 2*). Because of these findings the patient was referred to the Jewish Hospital X-Ray Department for a lateral mandibular plate picture, to check the possibility of generalized bone disturbance. The lateral plates revealed a complete loss of the trabecular structure, especially in the molar and premolar areas (*fig. 3*). The patient was questioned as to her history and related that she had been operated upon for osteomyelitis of the femur and had a splenectomy performed February, 1933. A

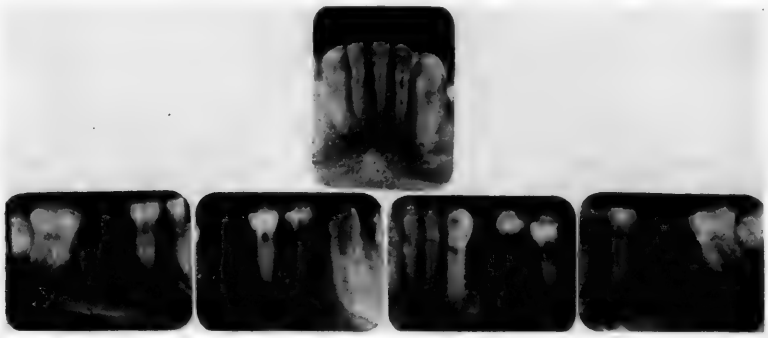

FIG. 1. Note cyst-like areas in left molar and premolar regions and absence of well defined wall as seen in true cysts. Lamina dura and peridental membrane are intact and sharply outlined. Note vacuolization between right mandibular premolars. Porosity of bone is well exemplified in mental and right canine region. Dark spot in center is genoid tubercule.

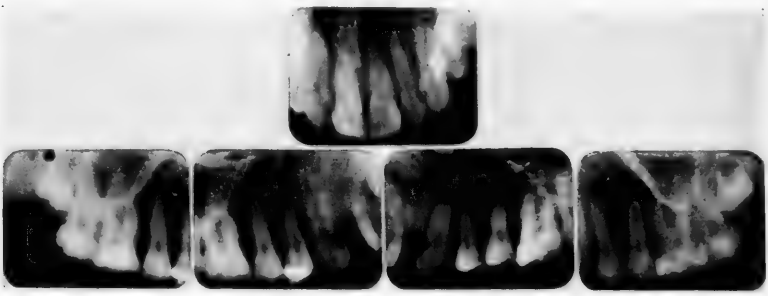

FIG. 2. Right premolar area clearly shows region of osteolysis. V shaped, radiopaque image is artefact.

search of the hospital record disclosed that the patient had been suffering with Gaucher's disease and the original diagnosis of osteomyelitis was in error. A study of her old x-rays revealed the trabecular destruction in the remainder of the skeleton and showed some of the associated bone lesions (*figs. 4 and 5*).

Case 2: A Jewish woman, age 27 years was sent to the Jewish Hospital X-Ray Out Patient Department on July 27, 1935 for a roentgenogram of her left humerus which she

fractured June 16, 1935. The x-ray revealed a fracture with unusual osseous changes. Because of this finding she was advised to enter the hospital for further study. The examination was essentially negative. There was no splenic or liver enlargement.

Laboratory data:—Hemoglobin 78 per cent, red blood cells 3,850,000, white blood cells 6,100, neutrophils 62 per cent, lymphocytes 35 per cent, eosinophils 3 per cent, blood calcium 10.0 mgs. per 100 cc. of blood, blood phosphorus 4.7 mgs. per 100 cc. of blood, blood cholesterol 147 mgs. per 100 cc. of blood, Bence-Jones, bodies test neg.

Roentgenograms revealed involvement of both humeri. The right humerus showed small areas of bone lysis irregularly distributed throughout cortex and medulla, and destruction of the periosteum on the mesial aspect about one inch below the head of the humerus. About the distal portion of the shaft of the bone was some medullary widening and this was accompanied by a thinning of the cortex. The lower end of the humerus

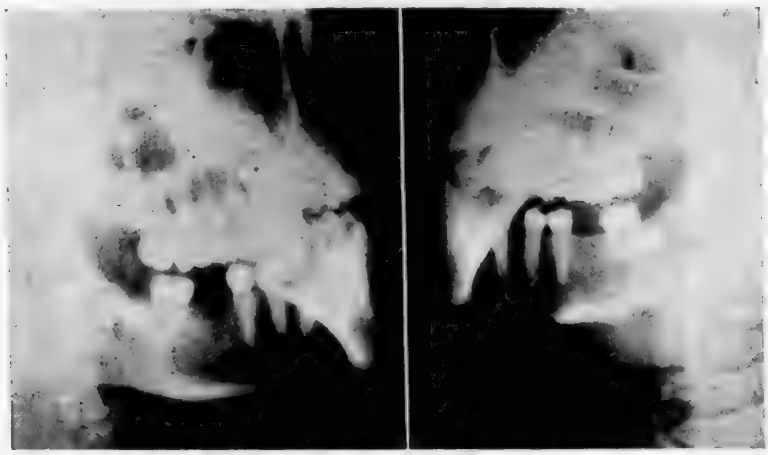

FIG. 3. Right and left side of mandible show a complete loss of trabecular structure in premolar zones. Note demineralization as it extends toward second molar area. There is some evidence of thinning of cortex.

had a trabeculated appearance. In the shaft of the humerus the usual transverse bony trabeculi were not seen (*fig. 6*). In addition to these changes there was a large area of rarefaction in the distal end of the right femur and in other parts of the skeleton. The general impression was that the bony changes were due to a metabolic disturbance most likely xanthomatosis or possibly Gaucher's disease. At the same time multiple myeloma must be excluded as well as lymphoid disturbances.

Because the lesions appeared in the cortex of the bone and there was some periosteal destruction and since Pick (9) and others are of the opinion that the periosteum and cortex are never invaded in Gaucher's disease, the latter seemed doubtful. The patient was referred to the dental department for further study. Oral examination revealed no inflammation of the mucuos membranes. The gingivae were slightly inflamed and puffed around the upper right canine and premolar areas, these changes were probably due to

a faulty filling. Some exudate could be elicited on pressure from the labial aspect of the lower anterior teeth. The remaining gingivae had a normal appearance. The floor of the mouth and the hard and soft palate were essentially negative. Intra-oral roentgenograms showed a generalized porosity in the mental region (*figs. 7 and 8*). Areas of bone lysis were noted especially in the right molar and premolar mandibular areas. The maxillary region showed bone lysis in the region of the upper left premolar area, while the canaliculi in areas of the upper third molars were enlarged. The upper left canine was impacted. Generalized porosity and demineralization was also noted in the maxillae.

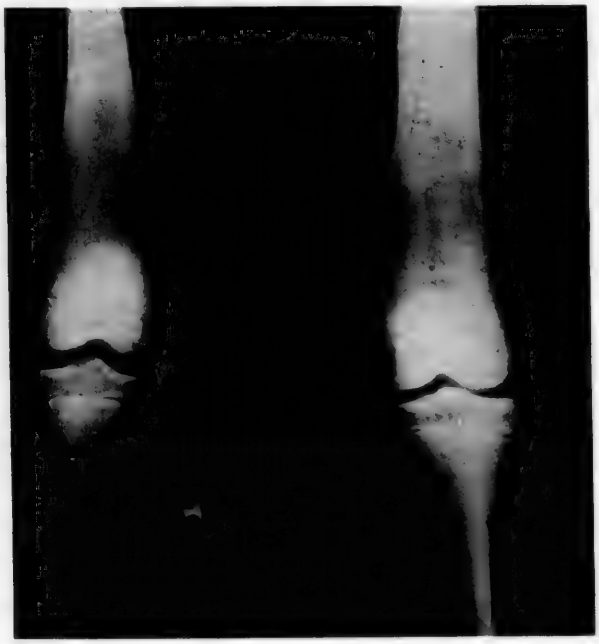

FIG. 4. Note clublike appearance of femur. This sign is of diagnostic significance in Gaucher's disease.

The peridental membrane and lamina dura were indistinct in the lower left premolar teeth. The pulp chambers were not enlarged. The upper right second molar was devitalized but did not have any periapical involvement. With the exception of the right second maxillary molar, all teeth responded to the electric pulp tester within normal limits.

The lesions found on dental x-ray examination in this case were similar to those in Case 1. A tentative diagnosis of Gaucher's disease was made. This diagnosis was confirmed at a later date by a biopsy taken from the bone cortex of the right humerus (*fig. 9*).

COMMENT

Although a diagnosis of Gaucher's disease was made prior to the dental findings in the first case, it is significant that the dental intraoral films aroused a suspicion of the presence of a constitutional disease. This was based principally on the presence of cyst-like areas of radiolucence in the molar and premolar mandibular region. A generalized porosity was also noticed in the mental region. Areas

FIG. 5. Shows demineralization in shafts of femur and areas of lysis

of radiolucence are often encountered under normal conditions particularly in the mandibular molar areas. Pollia (16) cited an instance of normal radiolucence that was mistaken for a cyst in the mandibular molar area. In cases of Gaucher's disease areas of radiolucence are often encountered and may sometimes be overlooked as a normal finding whereas actually they are pathologic conditions.

The patient in Case 2 was referred to the dental department for

dental study because of the findings in Case 1. This finding added more efficacy to a tentative diagnosis of Gaucher's disease because at the time multiple myeloma, possible malignancy and lymphoid disturbance were other factors to be considered. A biopsy of bone revealing presence of Gaucher's cells in the bone cortex established

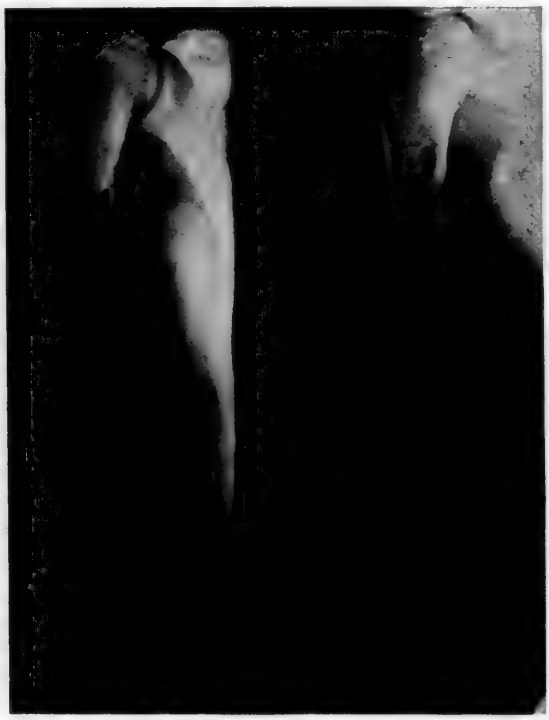

FIG. 6. Note areas of vacuolization in the medulla and cortex of the humeri. Of special interest is the subperiosteal erosion in the middle and proximal portions of the shafts. The above changes are produced by the infiltration of Gaucher's cells.

the definite diagnosis of Gaucher's disease. Another significant fact in Case 2 is that the presence of Gaucher cells in the bone cortex is reported for the first time both in medical and dental literature. The lesions found in the patient in Case 2 are at variance with such viewpoints as are held by Pick (9), Junghagen (12) and Fisher (13),

that the Gaucher's cells are never found in the bone cortex and the periosteum.

The author draws no conclusions but merely points to the constant presence of bone lysis in both cases. These findings may have some

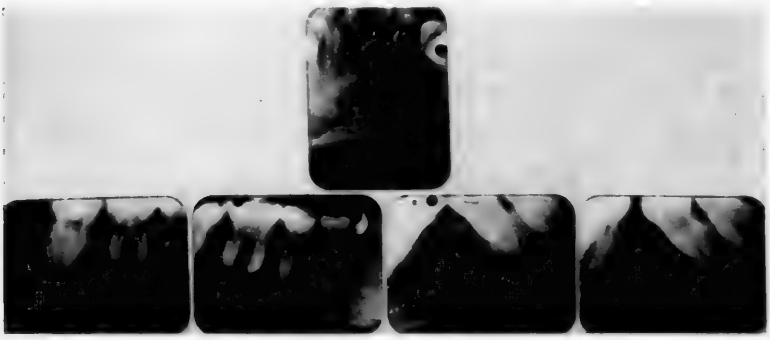

FIG. 7. Note porosity and moth eaten appearance in mental region. Molar and premolar zones show large areas of rarefaction with few strands of bone tissue still intact. This is probably due to slow infiltration of Gaucher's cells. Note slight erosion of lamina dura and peridental membrane in left premolar region. Large cystlike area with foamy appearance can be discerned between second left premolar and mesial root of first molar. Right molar area shows asymmetrical cancellations.

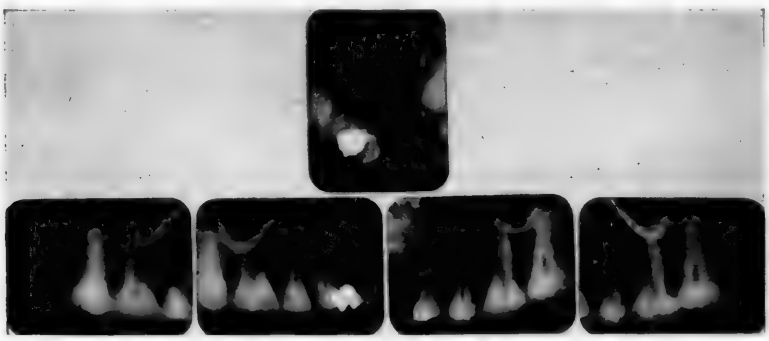

FIG. 8. Right premolar region shows areas of bone lysis, same as in Case 1. Canaliculi are enlarged in region of tuberosity. This is not to be regarded as pathologic.

diagnostic significance. Dental x-ray findings in Gaucher's disease show a generalized porosity with areas of radiolucence that are pseudo-cystic in appearance in the mandible and areas of lysis in the

maxillary premolar region. A differential point to consider is that radiolucent zones present in the mandible under normal conditions occur on both sides and are uniform in rarefaction. The bleeding of the gingiva (which is of especial interest to the dentist and oral surgeon) and nose, which is characteristic of the late stages of the disease is not due to the Gaucher's disease as is believed by Prinz and Greenbaum (17). It is mainly an expression of an associated thrombocytopenia. Breuer (18) cited 4 cases of Gaucher's disease with peridental involvement. The changes were part of the general

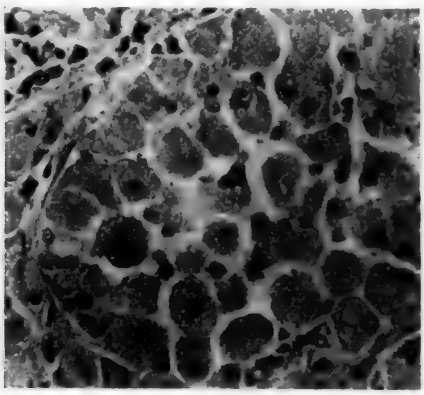

FIG. 9. High power: Shows nests of large Gaucher's cells possessing eccentric nuclei and considerable clear protoplasm. Most of cells possess one nucleus; a few are multinucleated. Cells are fairly uniform in size.

systemic reaction and did not represent a separate peridental involvement.

Since Gaucher's disease is chronic in its course and the bone lesions may be present even before splenic or liver enlargement as is shown in Case 2, the disease may be detected in its early stages by means of the dental x-ray.

SUMMARY

1—Two cases of Gaucher's disease with lesions in the mandible and maxilla are described. 2—In the first case, the disease was recognized by intra-oral films. 3—In the second asymptomatic case, the dental roentgenographic findings aided the establishment of a tentative

diagnosis of Gaucher's disease. 4—The presence of Gaucher's cells in the bone cortex and periosteum is reported for the first time. 5—Normal areas of rarefaction of the mandible differ from pathologic in that the pathologic, both sides are not uniform. 6—Generalized porosity in the mental region, pseudo-cystic areas of radiolucence in the molar and premolar mandibular region and presence of bone lysis in the maxillary premolar areas, are the three constant findings in the above two cases. 7—Bleeding from the gingiva is only present when there is an associated thrombocytopenia. 8—The dental findings in the above two cases may have some diagnostic significance.

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BONE IN PULP CHAMBER[1]

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For many years reports showing bone in the pulp chamber have been published. *Fig. 1*, a cross section of a lower molar, is representative of this condition. The entire pulp chamber is filled in with bone, in most places united with the dentinal wall. In other places large marrow spaces containing fat and islands of hemopoietic cells are present. In those areas where bone is being actively formed, adult connective tissue is found. The embryonic connective tissue characteristic of the pulp is entirely lacking.

Fig. 2 is a radiograph of a retained maxillary right deciduous canine in a patient 19 years old. *Fig. 3* is a low magnification of the apical end, repaired with secondary cementum. Secondary cementum also lines the walls of the root canal at SC. In the center of the canal a bony process (AB) has been developed. Adult connective tissue fibres are attached to the new bone and to the secondary cementum lining the canal in the characteristic manner of a periodontal membrane (PM). At P is normal pulp consisting of embryonic type connective tissue. *Fig. 4* is a higher manification showing the secondary cementum (SC) lining the canal, the new bone (AB), the new periodontal membrane (PM) and the pulp (P). This specimen confirms an observation (1) of a peculiar type of repair of a fractured tooth (*fig. 5*). This photograph is also of the canal, showing secondary cementum (SC) lining the canal, a new bony process (AB) and a new periodontal membrane (PM).

In both teeth shown in *figs. 3* and *5* there is evidence of a remarkable reparative power of adult connective tissue in an endeavor to maintain good functional activity. In these teeth there existed a wide

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space in the canal. Adult connective tissue from the periodontal membrane invaded the embryonic pulp tissue when the repair changes began. Embryonic pulp tissue is necessary for dentin formation. Adult connective tissue does not form dentin, but is capable of producing an attachment apparatus consisting of cementum, periodental membrane and bone. Where the space is wide mere deposition of secondary cementum on the walls of the canal, such as that found in repair following root canal therapy, is inadequate. To

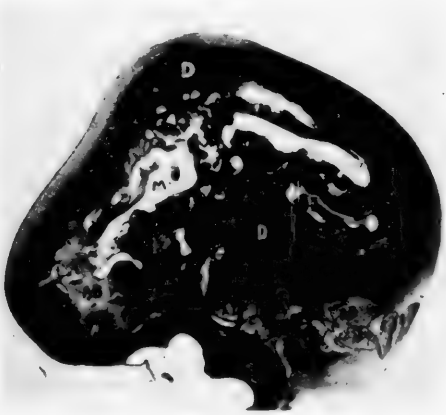

FIG. 1 FIG. 2

FIG. 1. AB, bone. D, dentin. M, marrow space

FIG. 2. Radiograph of retained right maxillary deciduous canine

maintain good functional activity new bone, continuous with the alveolar bone of the tooth socket, is built within the canal.

Where the space is narrow, as in root canal therapy and in some repaired fractures, secondary cementum lines only the exposed dentinal surfaces. This is well illustrated in *figs. 6* and *7*. *Fig. 6* shows partial repair of a tooth fractured 2 months before extraction. The periodontal membrane is present at PM. The narrow space between the separated fragments is shown at S. Adult connective tissue from the periodontal membrane has invaded this space and is already depositing secondary cementum (SC) on the dentinal surface. In the pulp chamber (P) secondary cementum (SC) is being

built on the pulpal wall. This is additional support for the statement that when adult connective tissue invades the pulp it will produce either cementum or bone but cannot produce dentin. *Fig. 7* is a photograph of a tooth extracted 12 years after root canal therapy. Here also, we see a narrow space (the former canal) lined with secondary cementum (SC).

There is a relation between bone in the pulp and internal resorp-

FIG. 3

FIG. 4

FIG. 3. AB, bone. D, dentin. P, pulp. PM, periodontal membrane. SC, secondary cementum.

FIG. 4. AB, bone. D, dentin. P, pulp. PM, periodontal membrane. SC, secondary cementum.

tion of dentin, (2–10). *Fig. 8* is a radiograph of a maxillary right second premolar showing internal resorption. *Fig. 9* is a low magnification of this tooth. At AB it can be observed that bone from the alveolus has grown through the tooth in a mesio-distal direction. In some areas there is ankylosis of the new bone with the dentinal surface while in other areas the dentinal wall is lined with secondary cementum. New periodontal fibres are connected from the new cementum to the new bone. This new bone contains marrow spaces,

fat and islands of hemopoietic cells. In this case bone is growing from the alveolar process horizontally into the tooth from the mesial and distal sides. Resorption in the canal—the first stage of repair

FIG. 5. AB, bone. D, dentin. PM, periodontal membrane. SC, secondary cementum.

FIG. 6. P, pulp. PM, periodontal membrane. S, space between fragments. SC, secondary cementum. SC′, secondary cementum in pulp.

following root canal therapy—continued until lateral perforations were made. Following the perforations adult connective tissue from the periodontal membrane invaded the canal. Here again, as in the teeth shown in *figs. 3* and *5*, the periodontal membrane demon-

strates its potential powers of being able to build either cementum or bone. In this instance, because the space was wide, bone was formed. It is logical for the new bone in *fig. 9* to act as an alveolar process even though its direction is horizontal. In *figs. 3* and *5* the

FIG. 7. D, dentin. SC, secondary cementum

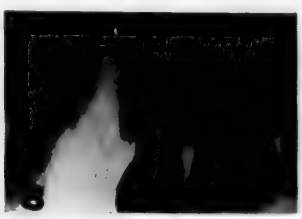

FIG. 8. Radiograph of maxillary right premolar with internal resorption

new bone is vertical. Development of bone in a horizontal direction is well shown in the wide separation space between fragments of a fractured tooth reported by Kronfeld (11).

Fig. 10 is a low magnification of a tooth showing internal resorption. The apical end was fractured during extraction and was not

replaced during sectioning because of uncertainty of establishing correct relationship. At B new bone is developing. In *fig. 11* a higher magnification of area B from *fig. 10* is shown. This new bone is being built from the adult connective tissue which invaded the pulp when lateral preforations followed the resorption process.

Fig. 12 shows two radiographs of another case of internal resorp-

FIG. 9

FIG. 10

FIG. 9. AB, bone. D, dentin. M, marrow space containing fat and hemopoietic cells

FIG. 10. B, developing bone

tion. The earlier radiograph (left) shows areas of resorption on both the mesial and distal sides. This tooth has a history of being severely traumatized on two occasions. The later radiograph (right) shows the tooth just before extraction. This is good evidence that in this case the internal resorption followed the external resorption which started from the sides of the tooth. *Fig. 13* shows perforation on the mesial side. Bone (AB) has grown into the center of the perfora-

tion as an auxiliary alveolar process, again in a horizontal manner. Secondary cementum has not yet had time to line the dentinal walls

FIG. 11. B, developing bone. P, pulp which has been converted to adult connective tissue.

FIG. 12. Radiographs showing internal resorption

(D), nor has the connective tissue (CT) had time to organize as a periodontal membrane. However, at B the bone has united with the dentin. In another area of this tooth (*fig. 14*) the embryonic pulp

tissue (P) has been changed to adult connective tissue. New dentin, therefore, could not be built, but there is evidence at B that new bone (intramembranous) is being formed, again supporting the statement that adult connective tissue has potential powers of building cementum or bone.

FIG. 13

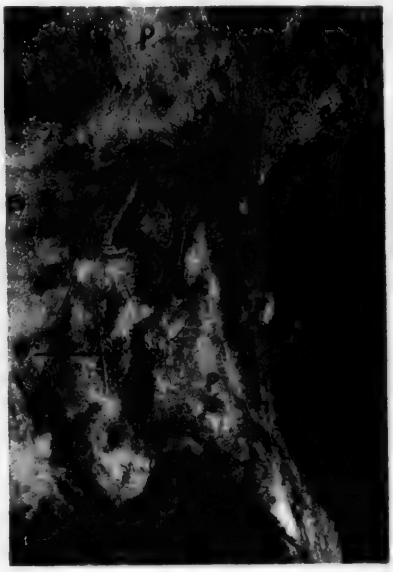

FIG. 14

FIG. 13. B, bone. D, dentin. CT, adult connective tissue fibers which have not had time to organize as a periodontal membrane.

FIG. 14. B, developing bone (intramembranous). D, dentin. P, pulp which has been converted to adult connective tissue.

CONCLUSION

When adult connective tissue (periodontal membrane) invades the pulp through a fracture, following root canal therapy or as a result of resorption, it exhibits its potential powers of building secondary cementum or bone. Adult connective tissue is incapable of building dentin. Where the space is narrow, secondary cementum repairs the dentinal surface. Where the space is wide, bone with new periodontal fibres attached to newly deposited secondary cementum on the

dentinal surface will result. In some cases ankylosis occurs, especially when the tooth is in good functional activity.

The author is indebted to Dr. M. Soifer for the specimen shown in *fig. 10*, and to Dr. M. Eggnatz for the specimen shown in *fig. 12*.

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THE BUCKY (GRENZ) RAY IN THE TREATMENT OF ORAL TUBERCULOUS LESIONS AND PERIODONTOCLASIA[1]

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In the field of oral tuberculosis we have been confronted with the problem of treating tuberculous ulcers involving the mucous membranes of the cheeks, lips, oro-pharynx; the superior and inferior maxillae, the floor of the mouth, and the tongue. There has been no generally accepted therapeutic measure for eliminating or even adequately controlling these tuberculous involvements. The lesions are usually highly resistant and not amenable to various chemical methods of therapy. The electrocautery is a useful adjunct in the treatment of some cases, but can not be considered an adequate therapy. Likewise quartz-lamp (ultra-violet therapy) has been used but the results are most ineffectual. Therefore, about a year and a half ago arrangements were made for the use of the Grenz ray apparatus which was adequately adapted for oral therapy, and shortly thereafter investigation was instituted.

Roentgen therapy, inspired by Freund, Senn and others, was instituted in 1896 as a result of loss of hair and the production of inflammatory responses in the tissues to the roentgen rays. Until 1902, roentgen therapy centered about the study of the skin. Subsequently, deep therapy was investigated, and this was followed by the treatment of internal diseases. The super-soft roentgen rays (Frank Schultz) appeared in 1910 and remained in use for about five years. In dermatology their use was found to be disadvantageous in that they produced telangiectatic and delayed atrophic conditions. Not much, however, was known of dosage or the quality of the rays. The same

[1] Read at a meeting of the New York Section, the International Association for Dental Research, June 2, 1938.

[2] This work was done in collaboration with Gustave Bucky, M.D.

was true in the misuse of the roentgen rays—perhaps even to a higher degree—with the appearance of undesirable sequelae. In view of the fact that the therapeutic effect of the super-soft rays differed from that of the familiar roentgen ray, the determining reasons had to be ascertained. This led to Bucky's investigations which culminated in what is now known as the Bucky Grenz ray. He conceived the idea of inserting a transmitting Lindemann window (a glass composed of the low atomic weight elements, lithium and boron) into a constant Coolidge tube with a voltage of only a few kilovolts, and he therewith introduced into radiation therapy soft radiation beams of great constancy, the biological effects of which differ from those of the shorter roentgen rays or of the longer ultraviolet rays.

The Grenz ray, which is a series of electromagnetic oscillations having a wave length of approximately two Ängstrom units, permits a diversification of dosage in the individual strata of the tissues, thus making possible absorption of the ray where it is most desired, without penetrating the deeper structures. At a depth of 3 mm. of cutaneous tissue with this wave length of two Ängstrom units, 90 per cent of the ray will be absorbed.

"The skin is the natural receptor for all sorts of irradiation. In this respect it subserves a rôle similar to that of special sense organs, namely the transformation of electromagnetic oscillations into other forms of energy (nerve stimulation, chemical reactions, et cetera). Even if this view must needs still be hypothetic, nevertheless numerous observations support it. This particularly applies to light, ultra-violet and Grenz rays. Light and ultra-violet rays have a limited depth effect, which by and large is not variable, at least not to so great a degree as that of the Grenz rays. This variability of penetration happens to be of unique interest as to the skin since this tissue has a stratified structure. It may be assumed that the upper layers of the skin depend in large measure upon the lower ones for their regeneration and reconstruction. Little is known of the specific rôle of each level. Pigmentation has been splendidly studied by Bloch and others. Its relation to the effects of light is understood. Bloch's illustrations of the nerve distribution are significant, showing the penetration of single fibrils to the very surface of the epidermis. These details serve to show how important it is to have at our disposal an energy affecting the skin, the deep absorption of which is capable of systematic variation. The Grenz Ray is this sort of energy, one which at will can be modified so as to supply specific stimulation both as regards depth and intensity." (1)

Thus it can be seen that this localization of the ray is a very important factor in its therapeutic use.

With the application of the Grenz ray a photo-chemical action is

instituted. This exerts apparently a stimulating effect on the tissue cells. As a result of a study of the effect of the ray on the eyes of rabbits, Thompson, Pfeiffer and Salardo (2) have reported a definite increase in anti-body formation subsequent to Grenz radiation.

It has been observed that tissues which have previously received roentgen rays or other forms of radiation treatment do not as a rule respond well to Grenz radiation. The other radiation apparently changes the tissue cells so that they are not satisfactorily receptive to the Grenz ray.

Tuberculous lesions involving the mucous membrane in the mouth are not only ordinarily highly resistant but are frequently extremely painful and not at all amenable to therapies usually applied to oral mucous membrane infections. In the treatment of these lesions with the Grenz ray, we not only observed arrest and gradual healing of the tuberculous processes, but where painful lesions existed, there was an almost miraculous disappearance of the painful manifestations within twenty-four or forty-eight hours after the first radiation.

The treatment of these patients consisted in the use of a small portable Grenz ray apparatus with a small easily manipulated Grenz ray tube at the end of which was placed a glass applicator. These glass applicators were so designed as to permit their being removed for the purpose of sterilization after each application. Their apertures vary in diameter so that they can be applied to lesions of all sizes in the mouth. Patients have been radiated on the average of once each week excepting those who were given an unusually high dosage. There were no discernible erythematous or other disturbing manifestations subsequent to our irradiations.

Following are some of our case reports:

Case I. Patient: W. M., male, negro, age 46. *Medical History:* Admitted 2/11/37; chronic pulmonary tuberculosis, tubercular ulceration of the tongue and lip. Subsequently developed cheek lesions. Gaffky—neg. Wasserman—neg. *Examination:* In July the patient presented with an extensive ulceration involving the anterior half of the tongue, another deep penetrative crater-like ulcer of the mucous membrane of the left cheek, and several smaller ulcers on the mandibular alveolar and lip membranes. Patient could open mouth for a distance of 1 cm. with difficulty and marked pain. Any digitation of the lesions was extremely painful.

Treatment: All therapies applied (chemical) were entirely ineffectual. He received his first Bucky-Grenz radiation July 9, 1937. Twenty-four hours later, he was free from pain and was able to open his mouth with considerable facility. Subsequent to 7

radiation treatments, his tongue lesions were completely healed, and the other lesions were free from inflammation and manifested very marked improvement. His general condition had likewise greatly improved, and he left the hospital. There is a tendency for hematogenous involvement, but as new lesions are about to form, our radiations either abort them or keep them within control. Several of these lesions formed and quickly healed.

The following schedule indicates the dosages administered:

KV	*MA*	*Dist.*	*R*	*Time*
7.5	8	4 cm.	1845	3 min.

After the first radiation treatment of a lesion, the R was reduced to 922.5 with occasional variations.

Case II. Patient: W. W., male, white, age 49. *Medical History:* Chronic pulmonary tuberculosis, tubercular osteomyelitis of the jaw (?), potential bilateral lesions. Gaffky—VI. Wasserman—neg. Edentulous.

Examination: In June 1937, radiographically there was no evidence of a bony involvement in the mandible. There was, however, a deep, crater-like ulcer of the mandibular mucous membrane in the region of the left canine to first molar. A biopsy of the marginal tissue was reported as granulation tissue. A second biopsy, of the deeper tissues, was reported as tuberculous granulation tissue.

Grenz Radiation History:

		KV	*MA*	*Dist.*	*R*	*Time*
6/30/37	Mandibular ulcer	7	8	3 cm.	2300	2½ min.
7/22/37	" "	7	8	4 "	1035	1½ "
8/ 5/37	" "	7.5	8	4 "	922	1½ "
8/19/37	" "	7.5	8	4 "	922	1½ "
9/ 2/37	" "	7.5	8	4 "	922	1½ "
9/30/37	New lesion	7.5	8	4 "	922	1½ "

Old lesion completely healed.

New lesion 1 cm. distal to old lesion formed.

10/28/37 New lesion completely healed.

The patient now wears two dentures and is entirely free from any oral disturbance.

Case III. S. D. Female, negro, age 19. *Medical History:* Admitted 10/18/34. Chronic pulmonary tuberculosis, tuberculosis of the larynx, pneumothorax of the right lung. Gaffky—Positive until 10/35. Negative since 10/35. Wasserman—Negative. *Dental History:* 10/29/36—Patient presented with tuberculous ulceration about 1½ x 1½ x 2 cm. on the labial maxillary mucous membrane in the region of the left first and second incisors which were missing. Bridge involving this region was removed at the first visit 6/11/35 and a biopsy taken. Laboratory report: tuberculous granulation tissue. Radiographs 6/11/35 revealed rarified bone in the area of the lesion. At present the lesion manifests a definite bone regeneration. At no time was the pain severe. *Treatment:* Ultra-violet therapy for approximately one year, electro-cauterization and localized medication were administered. There was no appreciable change as a result of these treatments in the size or character of the lesion. Following cauterization, the lesion developed hypergranulation tissue. After receiving five Bucky-Grenz radiation treatments, there was evidence of a marked reduction of the inflammatory process. With

continuation of these treatments, the lesion gradually completely disappeared, leaving a cicatrix which is scarcely visible. The dosages administered were similar to those of the previous mentioned case.

A group of more than fifty adult patients who manifested extensive suppurative periodontoclasias (some of the horizontal and some of the vertical types) received Grenz radiation treatments. As this was purely experimental, the dosages were considerably modified and the number of treatments was varied in different patients. Some of the patients received sub-gingival scalings prior to or during the course of radiation therapy, while others received nothing but Grenz radiations. In all cases, irrespective of methods employed, there was a definite evidence of a curative effect as a result of the Grenz radiations.

DISCUSSION

In a group of patients who received Bucky-Grenz radiations for the treatment of tuberculous ulcers involving the mucous membranes of the tongue, lips, cheek, soft palate, alveolar mucous membrane, and the posterior pharynx, each patient manifested a definite improvement or cure of the lesion treated. In some cases, the tuberculous spread involving other organs had become so advanced that the patient expired before he could receive sufficient Grenz radiation therapy.

It is anticipated that other surface tubercular lesions to which the treatment can be directly applied will respond equally well. In a large series of tubercular patients manifesting chronic (non-specific) periodontal lesions, Grenz radiation therapy to the gingivae resulted in marked diminution or complete removal of the suppurative or inflammatory sub-gingival processes.

CONCLUSIONS

1. Patients manifesting oral tuberculous ulcers involving the mouth, lips or oro-pharynx and who received either chemical, surgical or ultra-violet therapy (or combinations of these therapies) manifested, as a rule, little or no appreciable improvement in the lesions so treated. 2. Where these lesions received Bucky-Grenz radiations, there was manifested evidence of either a definite healing process, or

complete disappearance of the lesion. 3. Where painful lesions existed, the pain was nearly always either completely mitigated or else markedly reduced twenty-four to forty-eight hours after the first radiation treatment. 4. In a group of more than fifty adult patients who manifested extensive generalized suppurative periodontoclasia, Grenz radiation therapy effected a definite cure in each case.

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STUDY OF THE INFLUENCE OF MAGNESIUM AND SODIUM ON THE ACTIVITY OF FLUORIDES

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The investigations of Smith, Lantz, and Smith ('31), Smith and Smith ('32) and Dean ('35, '37) furnish evidence that fluorine, occurring as fluorides in water used for drinking and cooking purposes, is the chief etiological factor in mottled enamel. The toxic effect of fluorides appears to be uninfluenced by dietary factors (Smith, '36). Because of their great importance as tooth-forming elements, calcium and phosphorus have received special attention in this connection. Variations in the calcium and/or phosphorus content of the diet have been found to be without effect on the action of fluorine (Dean '34, Smith '36). On the other hand, Dean ('35, '36) considers that the activity of fluorides may be affected by other constituents of the water, or some yet undetermined factor.

The experiments to be reported were undertaken to investigate two of the common constituents of water, magnesium and sodium, in relation to their effect upon the toxicity of fluorides, with the albino rat as the experimental animal. So far as is known to the author, there have been no published reports of studies in this field.

EXPERIMENTAL PROCEDURE

Three types of water were used for the first study: city water of Lubbock, Texas, which has been shown to have a mean annual fluorine content of 4.4 p.p.m. (Dean '37); the same water which had been run through a zeolite softener; and distilled water. The zeolite treatment of water results in replacement of calcium and magnesium with sodium, but chemical analysis indicates that the fluorine content is not materially altered (Goodwin). Both the original city water and the zeolite treated water were concentrated to about $\frac{1}{10}$ the original volume, following the method of Smith, Lantz, and Smith ('31) for the experimental production of fluorosis in rats with water from an endemic area. Six male albino rats were placed at weaning on the stock diet (Sherman's Diet B). Two of the rats were given the concentrated city water, 2 the concentrated zeolite treated water, and 2 distilled water, the last named serving as controls.

Frequent comparison of the first two groups with the controls revealed marked changes in the teeth of both of the former in 3 to 4 weeks. The yellow color and the shiny translucent appearance of the normal incisors of the rat gradually disappeared. As time went on the teeth became white and chalky and the tips of the incisors were chipped and worn, all of which has been previously described as characteristic of dental fluorosis in the rat (Smith and Lantz '33).

The effect on the teeth was more marked with the zeolite treated water than with the untreated city water. This was probably due to the conversion of other fluorides present in the water to sodium fluoride, the latter having been shown to be the most toxic form investigated (Smith and Leverton '34). Since, however, both the magnesium and sodium content of the water was altered by the zeolite treatment, a further investigation of these two factors suggested itself. It was considered that calcium, in the light of the investigations previously cited, might be ruled out as a factor.

A second experiment was planned employing 6 different diets with composition as follows:

Diet I or Control Diet

INGREDIENTS	PER CENT BY WEIGHT	INGREDIENTS	PER CENT BY WEIGHT
Casein	18	Cod liver oil	3
Salt mixture O. and M.	4	Butter fat	7
Dried brewers' yeast	15	Cornstarch	53

Diet II

Same as I, with the addition of 0.6314 g. sodium carbonate per 100 g. ration, doubling the amount of sodium furnished by the Osborne and Mendel salt mixture alone.

Diet III

Same as Diet I with the addition of 0.2218 g. magnesium carbonate per 100 g. ration, doubling the amount of magnesium furnished by the Osborne and Mendel salt mixture alone.

Diet IV

Same as II with the addition of 0.1 per cent sodium fluoride (0.0452 per cent fluorine).

Diet V

Same as Diet III with the addition of 0.1 per cent sodium fluoride.

Diet VI

Same as Diet I with the addition of 0.1 per cent sodium fluoride.

Twenty-four albino rats 28 to 30 days of age, weighing 50 to 60 grams, were divided into 6 groups, each group being placed on one of the above diets. Distilled water was used in all cases. The results in terms of food consumption, growth and changes in the teeth during the first 5 weeks of the experiment are given in Table I.

The experiment was continued until the rats were 4½ months of age. At no time was there any evidence of changes in the teeth due to excess magnesium or sodium alone. As time progressed the incisors of the rats on the fluoride-containing diets showed corroding and pitting of the enamel, and the elongation which has been reported by others (Smith and Lantz '33) was also noted. It was not possible to detect by gross examination any differences in the effect of the 3 diets containing sodium fluoride. The excess of sodium in Diet IV or of magnesium in Diet V did not appear to lessen or increase the toxicity of the fluoride, using gross changes in the incisors as an indication of fluorosis. There was, however, a slightly higher food consumption on these diets than on Diet VI. This increased food consumption on Diet IV was accompanied by a gain in weight which exceeded that of the rats on Diet VI, but there was no appreciable difference in the latter respect between the rats on Diet V and Diet VI. It was thought that perhaps the greater gain on Diet IV might be attributable in part to an increased water retention produced by the sodium, but there was no evidence of a like effect of sodium in Diet II.

The problem of the maternal transference of fluorides, both placental and through the milk is one that has recently received considerable attention. Murray ('36) has presented evidence of maternal transference of fluorine both during uterine life and during lactation, with rats on diets containing 0.05 per cent sodium fluoride. On the other hand Phillips and Hart ('34) have found that a fluoride intake sufficient to produce a toxic effect in cows does not appreciably increase the fluorine content of the milk as measured by its effect on rats.

It was planned to investigate the 3 fluoride-containing diets from the standpoint of transference of fluorides to the young if it was found possible to secure reproduction. Failure to reproduce has been reported (Smith and Leverton '34) when sodium fluoride at 0.1 per cent

level was incorporated in Sherman's Diet B. At lower levels in which reproduction was normal the young were found to be less vigorous.

The rats in the control group as well as those on Diets IV, V, and VI were mated. A control female gave birth to a litter of 8 at 4 months and 12 days, and one of the females on Diet V gave birth to 6 young at 4 months and 14 days. Up to 5 months of age, when it became necessary to discontinue this part of the study, reproduction had not occurred in the other groups, so that in the end the only comparison possible was that of the control litter with the litter born of the mother on Diet V.

TABLE I

Food consumption, growth and changes in teeth of rats on different diets

DIET	NUMBER OF RATS	AVERAGE FOOD INTAKE—GMS.	AVERAGE GAIN IN WEIGHT—GMS.	CHANGES IN TEETH
No. I (Control)	4 2 males 2 females	329	97	None
No. II (High sodium)	4 2 males 2 females	322	95	None
No. III (High magnesium)	3 1 male 2 females	322	98	None
No. IV (No. II plus 0.1% NaF)	5 3 females 2 males	238	70	Loss of pigment Chalky
No. V (No. III plus 0.1% NaF)	4 2 males 2 females	245	50	Loss of pigment Chalky
No. VI (No. I plus 0.1% NaF)	4 1 male 3 females	217	49	Loss of pigment Chalky

The average weight of the young of the control mother at birth was 5.7 grams and that of the young of the mother on the diet containing excess magnesium and 0.1 per cent sodium fluoride was 5.1 grams. Two of the control litter and 3, or half, of the litter born of the mother on Diet V died shortly after birth. The surviving rats in both groups appeared vigorous at first, but there was a marked difference in the

rate of growth in favor of the control litter. With initial weights very close together there was at weaning time a wide variation in the weights of the animals of the 2 litters, which suggested that transference of fluorides through the milk was a larger factor than transference through the placenta. It is recognized, however, that failure to grow may have been in part the result of a deficient milk supply resulting from the lowered nutritional status of the mother. At weaning time the weights of the young of the control mother were 57, 59, 52, 54, 42, and 55 grams respectively; those of the young of the mother on Diet V were 21, 21, and 17 grams respectively. There was also a definite contrast, in the appearance of the teeth of the 2 litters before weaning time. Those of the control litter had a yellow tinge which deepened in color as the rats grew older. The teeth of the litter of the mother on Diet V were white and chalky in appearance, this condition becoming more marked as time went on.

Further studies on reproduction and the maternal transference of fluorides with different types of diets are in progress.

CONCLUSIONS

The toxicity of fluorides does not appear to be influenced by the amount of magnesium present in water and food.

There may be a relationship between the activity of fluorine in drinking water and the amount of sodium present, in that a high sodium content may mean that a greater part of the fluorine is present in the form of sodium fluoride, which has been shown to be more toxic than the other forms investigated.

The addition of sodium carbonate to a diet to which has been added 0.1 per cent sodium fluoride does not increase the severity of the dental fluorosis in the albino rat, so far as can be determined by gross examination of the incisors.

Reproduction with rats reared on diets containing as much as 0.1 per cent sodium fluoride may occur, even though such diets result in a lowered nutritional status, as evidenced by the growth stunting effect.

When reproduction does occur, the young do not grow normally, although the weight at birth may not deviate greatly from the normal. The latter observation suggests that transference of fluorides through the milk may be a larger factor than transference through the placenta.

The growth effect, however, may be the result of a decreased milk supply.

The incisors of the young of rats on diets containing 0.1 per cent sodium fluoride show evidence of fluorosis before weaning time.

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POSSIBLE RELATIONSHIP BETWEEN THE FLUORINE CONTENT OF ENAMEL AND RESISTANCE TO DENTAL CARIES[1]

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That the susceptibility and immunity of teeth to caries is to be accounted for, at least in part, by differences in composition is a hypothesis that has long been attractive. The evidence for the claims (7, 8) that such differences exist have failed of confirmation. We (3) found enamel and dentin fractions prepared from sound and carious teeth to possess compositions, with respect to the calcium, phosphorus, magnesium, and carbonate contents, which were not significantly different. LeFevre and Hodge (9) found whole tooth inorganic substance prepared from carious specimens to differ from that obtained from sound teeth only with respect to a slightly higher moisture content of carious tooth substance.[2]

Without regard to the data to be presented below, there is evidence that marked changes in some of the properties of enamel accompany changes in its fluorine content. The amount of fluorine in mottled enamel is relatively slight (4) in comparison to the total inorganic material; yet it produces, or is accompanied by, profound changes in the appearance and properties of the enamel. In this connection it is worthy of mention that it has not been demonstrated that mottling of enamel is due entirely to the effect of fluorine combined in the inorganic phase and is possible that the condition is produced, in part, by the operation of the fluoride ion on enzymes during the calcification of the enamel. The effect of fluorine added to the diet on the quality of the teeth of rats first observed by Schulz and Lamp (13) and independently by McCollum, Simmonds, Becker,

[1] Presented in part at the Baltimore meeting of The International Association for Dental Research, March 13, 1937 (*J. D. Res.*, 16: 309 (1937)). This work was supported by a grant from the Graduate Medical Research Fund of The University of Minnesota.

[2] See also French, Welch, Simmons, LeFevre and Hodge, *J. D. Res.*, 17: 401, 1938 (this issue)—Ed.

and Bunting (10) has since been noted by numerous workers. The hardness of the incisor teeth, which in the rat grow continuously throughout life, is augmented by increasing, within certain limits, the amount of fluorine in the diet so that they are not worn at the usual rate and therefore become much longer than those of normally fed animals. Such teeth may be regarded as possessing some superior qualities even though they may not be adapted to an efficient use by the rat and in spite of the fact that the calcification of the enamel and dentin may be abnormal. Armstrong (1) fed an adequate diet to rats to which was added during alternate three week periods 0.1 per cent sodium fluoride. Alternating transverse bands of mottled and normal enamel were thus produced across the incisor teeth. These teeth after attaining a considerably increased length frequently fractured, but the fracture line was usually transversely situated through a band of normal enamel. It thus appears that the hardness and strength of the incisor teeth of rats is improved by a moderately increased fluorine content of the mineral phase or by some change produced in the structure of the tooth which occurs during the formation of the enamel and dentin under the influence of a higher than normal concentration of fluorine.

The literature (11) contains occasional statements to the effect that moderately mottled human teeth are apparently more resistant to caries than normal teeth. Every dental clinician interviewed by us and who has seen numerous cases of mottled enamel expressed the opinion that the incidence of caries in such teeth, if the mottling is slight to moderate, is greatly reduced. A very carefully controlled study is needed to determine the exact differences in susceptibility to caries of mottled and non-mottled teeth.

METHODS AND RESULTS

The teeth were prepared for analysis, the enamel and dentin fractions separated and the fluorine determinations made by methods previously described (3, 2). The carious lesions were entirely ground away before the enamel and dentin fractions of the decayed teeth were separated. With a very few exceptions no restorative work had been applied to the carious teeth. The average difference in the age of the persons contributing the sound and carious teeth was approximately 10 years, the sound teeth on the average being obtained from older persons. All tooth types, except third molars, were studied in both groups. Most specimens of enamel were prepared from a single tooth but in 10 instances the enamel fractions of 2 or more sound teeth from one patient were pooled. Two specimens of enamel of carious teeth were prepared by pooling the enamel fractions of 2 teeth from one person.

DATA

The data displayed in Table I indicate the marked difference in the fluorine content of the enamel of sound and carious teeth of the same individual.

TABLE I

Fluorine content of enamel of sound and carious teeth of the same person

TOOTH	MALE AGE 36		FEMALE AGE 28		SEX AND AGE UNKNOWN	
	Sound Enamel	Carious Enamel	Sound Enamel	Carious Enamel	Sound Enamel	Carious Enamel
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
*	0.0160[1]					
$\frac{5}{}$ |		0.0087	0.0114		0.0130	
| $\frac{6}{}$		0.0065[2]	0.0095			
$\frac{6}{}$ |		0.0058				
| $\frac{7}{}$		0.0070[3]	0.0108			0.0056
$\frac{2}{}$ | $\frac{2}{}$			0.0115			
| $\frac{3}{}$			0.0110		0.0105	
$\frac{3}{}$ |			0.0117		0.0100	
$\frac{4}{}$ |			0.0112			0.0078
| $\frac{5}{}$			0.0108		0.0119	
$\frac{7}{}$ |			0.0105			
| $\frac{1}{}$				0.0066		
$\frac{1}{}$ |				0.0060		
$\frac{2, 1}{}$ |					0.0109	
| $\frac{4}{}$					0.0098	
| $\frac{4}{}$					0.0117	
| $\frac{5}{}$					0.0107	
$\frac{5}{}$ |					0.0110	
$\frac{4}{}$ |					0.0128	
Mean.....	0.0160	0.0070	0.0109	0.0063	0.0112	0.0067

* Pooled enamel of upper right 1, 2, 3, 4, 7 and upper left 7, 8.

[1] Results of repeated analysis: 0.0162, 0.0157, 0.0163, 0.0154, 0.0164, 0.0160.

[2] Results of repeated analysis: 0.0065, 0.0065, 0.0065.

[3] Results of repeated analysis: 0.0072, 0.0068, 0.0070, 0.0072.

Specimens furnished by Dr. Louie T. Austin, Mayo Clinic, Rochester, Minn.

The data in Table II are the results of 26 consecutive analyses. These results show that a low fluorine content is a characteristic of carious teeth in general and is not a quality which is observed only when the composition of the enamel of sound and carious teeth from the same individual are compared. The data also demonstrate that the quantity of fluorine present in the enamel of sound teeth is uni-

formly higher than that of carious teeth even though the specimens are procured from persons of various ages and from both sexes.

Nine carious teeth were obtained from a male, age 21. The other teeth had been extracted previously because of decay. The fluorine contents, in per cent, of the enamel fractions of the teeth available to us were as follows: $\underline{|1,2,}$ 0.0076; $\underline{|3,}$ 0.0087; $\underline{3|}$, 0.008; $\overline{|4,}$ 0.0054; $\underline{|4,}$ 0.0064; $\underline{|5,}$ 0.0054; $\underline{|6,}$ 0.0065; $\underline{7|}$, 0.0055. These results demonstrate that a low fluorine content characterizes the enamel of the carious teeth of a person suffering from rampant caries and is not restricted to the enamel of the carious teeth of persons possessing less than the usual number of caries.

TABLE II

Fluorine content of enamel of sound and carious teeth of several individuals

ENAMEL OF SOUND TEETH			ENAMEL OF CARIOUS TEETH		
Age and Sex of Patient	Tooth	Fluorine Content	Age and Sex of Patient	Tooth	Fluorine Content
		per cent			*per cent*
Male, 37	$\underline{1\|}$	0.0124	Female, 36	$\overline{7\|}$	0.0069
Female, 47	$\underline{\|5}$	0.0167		$\overline{3\|}$	0.0060
Male, 59	$\overline{4\|}$	0.0095	Male, 24	$\underline{\|5}$	0.0061
	$\underline{\|4}$	0.0091		$\overline{\|4}$	0.0078
	$\overline{2\|}$	0.0095	Male, 50	$\overline{6\|}$	0.0066
Female, 25	$\underline{\|5}$	0.0103	Male, 55	$\overline{7\|}$	0.0049
	$\overline{\|4}$	0.0093	Female, 44	$\overline{\|7}$	0.0073
Female, 13	$\underline{\|3}$	0.0099	Female, 18	$\underline{\|5}$	0.0064
Male, 47	$\underline{\|1}$	0.0107	Female, 18	$\underline{3\|}$	0.0073
Male, 74	$\underline{\|5}$	0.0131		$\overline{\|7}$	0.0073
Male, 70	$\underline{3\|}$	0.0133		$\overline{\|2}$	0.0072
Female, 44	$\overline{\|4}$	0.0111		$\underline{\|5}$	0.0080
Female, 37	$\underline{2\|}$	0.0136	Male, 37*	$\underline{\|4}$	0.0075
Mean		0.0114	Mean		0.0068

* The enamel of the sound $\underline{|1}$ from the same person contained 0.0124 per cent fluorine.

An assay of the rôle of composition of enamel in contributing to resistance to caries could be furthered by a study of the composition of the sound permanent teeth of very young persons in relation to known incidence of decay in the various tooth types. It has been difficult to obtain such teeth from youths. The enamel fractions of

the right and left maxillary first premolar teeth of a girl, age 10, were found to contain 0.0089 and 0.0081 per cent fluorine, respectively. The pooled enamel of the mandibular right first and second incisor teeth of a boy, age 9, contained 0.0101 per cent fluorine. The fact that a difference in the fluorine content of the enamel of newly erupted teeth occurs lends emphasis to our belief (3) that the composition of enamel is essentially determined at the time of eruption of the tooth.

Table III is a summary of all results.

TABLE III

Mean fluorine content of enamel and dentine of sound and carious teeth

	MEAN FLUORINE CONTENT	STD. DEVIATION OF MEAN	RANGE OF FLUORINE CONTENT	NUMBER OF ANALYSES
	per cent	*per cent*	*per cent*	
Enamel of Sound Teeth	0.0111	0.00203	0.0073[1]–0.0167	46
Enamel of Carious Teeth	0.0069	0.00111	0.0049–0.0087	39
Dentin of Sound Teeth	0.0169*			
Dentin of Carious Teeth	0.0158*			

* Samples prepared by pooling 50 mg. quantities of dentin from each of 26 teeth.

[1] Only one specimen of enamel from sound teeth was found to contain less than 0.0091 per cent fluorine.

DISCUSSION

The difference in the mean fluorine contents of the enamel of sound and carious teeth is 11.9 times its standard error. This figure indicates that the odds against the determined difference in the means of the two groups of data being due to chance alone is somewhat greater than 5,000,000 to 1, a probability so remote as to demonstrate that the difference is certain. There is no significant difference, however, in the fluorine content of the dentin of sound and carious teeth.

We are cautious as to the interpretation of the results of this study, and the position which we assume in regard to the obvious implications of these data is subject to alteration. Nevertheless, on account of the character of the results, the possible relationship between the fluorine content of enamel and dental decay should not be overlooked or minimized.

The low fluorine content of enamel of carious teeth does not appear

to be secondary to the carious process because the decayed enamel and dentin was entirely removed before the enamel fractions of these teeth were prepared. We have previously presented (3) evidence that decay of enamel does not produce alterations beyond the site of the lesion, in the calcium, magnesium, phosphorus, and carbonate contents, and there is no good reason to consider that the fluorine contained in enamel might be unique in this respect. The contention that the composition of enamel is not altered, except by decay, after the eruption of the tooth is fully substantiated by the evidence presented in the review by Schour (12). Aub (5) has recently shown that radium and radio-active lead do not enter the enamel of erupted teeth but are deposited in the dentine. One is to regard the samples of enamel and dentin of both the carious and sound teeth to have possessed the determined amounts of fluorine since the eruption of the teeth from which they were obtained. The low fluorine content of enamel of carious teeth, most probably not being secondary to the carious process, must therefore, *if associated with the condition in any manner*, bear a causal relationship.

It was necessary to remove the visibly decayed enamel and dentin before the carious teeth were prepared for analysis since to have proceeded otherwise would have resulted in the determination of alterations in composition which would certainly have been secondary to the carious process. Thus the material analyzed was really the sound enamel and dentin from carious teeth. How can it be explained, then, if a low fluorine content contributes to susceptibility to decay, that the lesion of caries is confined to one portion of the tooth crown and is not a diffuse process? It is well known that certain areas of a tooth are more apt to become carious than the so-called immune areas. The susceptible regions are the contact points, gingival margins, pits, and the occlusal fissures. It is not unreasonable to suppose that those factors which produce caries are able because of localized conditions of configuration of the tooth, stagnation of food debris, etc., to exert their maximum strength at the well known sites of the origin of caries. A tooth whose resistance to caries is reduced over its entire surface develops caries first at the site of those areas at which there is a local increase in the vigor of the caries producing factors or

because of a local decrease in the other factors which make for resistance to decay.

The fact that teeth exhibiting severe degrees of mottled enamel and containing a considerably increased amount of fluorine may become carious (4) does not argue against the hypothesis set forth above. It is well known (6) that the structure of the enamel of the more severely mottled teeth is altered so that it is brittle, develops pits and becomes eroded. For this reason it is possible that caries occurring in severely mottled teeth are not entirely comparable to caries of non-mottled teeth. The fact that the relatively slight amounts of fluorine in mottled enamel are accompanied by profound changes in its properties (4) make it seem entirely possible, in the light of the evidence presented above, that optimum quantities of this element in enamel might be associated with an increased resistance to caries without the deleterious effects on the appearance and the structure produced by an excessive fluorine content.

SUMMARY

The mean fluorine content of the enamel of sound and carious teeth has been found to be 0.0111 and 0.0069 per cent, respectively.

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CALCIUM, PHOSPHORUS AND CARBON DIOXIDE DETERMINATIONS ON ALL THE DENTIN FROM SOUND AND CARIOUS TEETH[1]

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INTRODUCTION

The recent development of a rapid quantitative method for the separation of enamel and dentin of the whole tooth (19) offers the opportunity for determination of the inorganic constituents in the separated tissues and for a comparison of these constituents on the basis of the extent of caries found in the tooth. Murray and Bowes (20) have analyzed a number of samples of separated enamel and dentin from sound and carious teeth. Their samples, however, do not represent the whole tooth for they were prepared by grinding from the enamel and dentin toward the dentino-enamel junction. Armstrong and Brekhus (1) using their flotation method for separation of the enamel and dentin have analyzed enamel and dentin from a number of sound teeth and the enamel of some carious teeth.

METHODS

Teeth[2] obtained from 3 full-mouth extractions were separated into enamel and dentin portions by LeFevre and Manly (15) according to a flotation method (19). On these samples of dentin and enamel, Ca, P, and CO_2 determinations were carried out as follows: Dentin samples weighing 150–250 mg. were dissolved in dilute HCl and made up to 100 cc. volume. Aliquots which furnished approximately 7 mg. of Ca were taken in duplicate for Ca determinations. The method used was a modification of the Clark and Collip (5) method by LeFevre and Nicholson (16). P determinations were run in duplicate according to Holtz's method (10). Single CO_2 determinations were made using the Shear and Kramer method (21).

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[1] This work was supported in part by a grant from the Carnegie Corporation of New York.

[2] Carious material is removed from the tooth with a dental burr before the enamel and dentine are separated for subsequent analyses.

TABLE I. PART A

Analytical data percentages based on inorganic weight

TOOTH NO.	TOOTH POSITION	CARIES CLASS	Ca PER CENT	P PER CENT	Ca:P PER CENT	CO_2 PER CENT
Patient E. R., Female, Age 41						
394	$\overline{1}$	0	—*	17.71	—	3.58
378	$\overline{2}$	1	35.77	17.63	2.03	4.03
379	$\overline{3}$	1	35.71	17.75	2.01	3.64
383	$\overline{4}$	1	37.33	16.48	2.27	3.95
382	$\overline{5}$	1	36.28	17.25	2.10	4.09
385	$\overline{7}$	2	34.49	17.72	1.95	3.89
380	$\underline{1}$	0	34.91	17.22	2.03	3.27
381	$\underline{2}$	2	35.48	18.00	1.97	3.09
389	$\underline{4}$	1	36.21	16.45	2.20	3.33
388	$\underline{5}$	1	36.09	17.96	2.00	3.64
386	$\underline{6}$	1	36.07	17.47	2.06	4.30
384	$\underline{2}$	2	34.18	16.81	2.03	3.56
387	$\underline{3}$	3	35.52	16.39	2.17	3.97
393	$\underline{4}$	3	35.82	17.29	2.07	4.18
392	$\underline{6}$	2	36.13	17.15	2.11	4.42
377	$\underline{2}$	1	34.35	16.01	2.15	4.06
395	$\underline{3}$	1	35.25	17.38	2.03	4.32
396	$\underline{4}$	1	35.52	17.58	2.02	3.65
391	$\underline{7}$	2	35.63	16.82	2.08	4.63
390	$\underline{8}$	3	36.16	16.76	2.16	3.99
Patient C. L., Male, Age 21						
309	$\overline{1}$	1	34.86	17.26	2.02	4.10
308	$\overline{2}$	1	—	16.46	—	3.64
307	$\overline{3}$	2	35.36	16.86	2.10	4.34
306	$\overline{4}$	2	36.19	16.57	2.18	4.19
304	$\overline{8}$	1	36.10	17.24	2.09	3.91
310	$\underline{2}$	2	35.80	17.03	2.10	3.85
311	$\underline{3}$	1	34.94	16.69	2.09	4.07
312	$\underline{4}$	2	34.01	17.12	1.99	4.62
313	$\underline{5}$	2	36.00	17.22	2.09	4.08
305	$\underline{6}$	3	35.49	16.71	2.12	4.21
314	$\underline{8}$	0	35.53	17.44	2.03	4.12
300	$\underline{3}$	2	35.52	17.12	2.08	4.22
299	$\underline{4}$	3	34.16	16.86	2.02	3.96
297	$\underline{7}$	1	34.99	17.78	1.97	4.44
302	$\underline{3}$	1	35.32	16.97	2.08	3.63
298	$\underline{7}$	2	33.42	17.34	1.93	4.72
303	$\underline{8}$	1	35.85	16.97	2.11	3.59
Patient J. L., Male, Age 17						
332	$\overline{1}$	3	36.41	17.06	2.13	3.98
331	$\overline{2}$	1	35.06	16.33	2.15	4.27
330	$\overline{3}$	3	35.20	16.67	2.11	4.11
329	$\overline{4}$	1	35.26	17.13	2.06	4.14
328	$\overline{5}$	2	35.37	17.44	2.03	4.63
327	$\overline{6}$	3	35.65	17.30	2.06	3.75

* This value (32.53) was discarded on the basis of the standard deviation.

TABLE I. PART A—*Concluded*

TOOTH NO.	TOOTH POSITION	CARIES CLASS	Ca PER CENT	P PER CENT	Ca:P PER CENT	CO_2 PER CENT
Patient J. L., Male, Age 17—*Continued*						
321	1	3	35.06	16.65	2.11	2.88
320	2	3	35.83	17.25	2.08	3.26
319	3	3	36.43	17.29	2.10	3.84
318	4	2	34.51	16.51	2.15	4.27
317	5	1	35.50	17.66	2.01	3.98
316	6	2	34.50	16.83	2.04	4.69
315	8	0	35.06	17.13	2.05	3.74
337	3	0	33.33	16.44	2.03	3.71
336	5	3	35.57	17.24	2.06	4.37
335	6	2	35.41	16.80	2.10	4.65
322	7	2	34.13	17.13	1.99	4.13
333	8	0	35.38	17.48	2.02	3.74
326	3	3	35.15	17.55	2.00	3.96
325	4	3	35.32	16.85	2.10	3.98
324	5	3	35.33	17.05	2.07	3.70
323	6	3	35.00	16.66	2.10	4.11
334	8	3	35.30	17.53	2.01	3.63

DATA

The analytical data for the dentin of 60 teeth are given in Table I, part a. The 3 patients, E. R., C. L. and J. L. provided 20, 17 and 23 teeth, respectively. The analytical data determined directly were percentages of Ca, P and CO_2 on the basis of inorganic weight. In addition to the analytical results, Table I also shows the tooth position, the caries class, the age and sex of the patient, and the Ca:P ratios.

The percentages of Ca, P and CO_2 on the basis of moist weight were calculated from the above analytical data using the per cent of inorganic moist weight of dentin as determined by LeFevre and Manly (15). Since no additional information was obtained, these figures were omitted. The ranges for percentages of Ca, P, CO_2 and Ca:P ratio for each patient separately and the over-all ranges for each constituent are given in Table I, part b. This part also gives the average percentages for Ca, P, Ca:P ratio and CO_2 for each patient; the general average for all the teeth and the respective standard deviations.

In Table II, part a, the average percentages for Ca, P, Ca:P ratio and CO_2 are listed for each patient in order of severity of caries; part b, gives the general averages for each constituent in the various caries classes.

TABLE I. PART B

Summary of analytical data

PATIENT	NO. TEETH	Ca			P			Ca/P PER CENT			CO_2		
		Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
E. R.	20	37.33	34.18	35.63*	18.00	16.01	17.19	2.27	1.95	2.08*	4.63	3.09	3.88
C. L.	17	36.19	34.01	35.22†	17.78	16.46	17.03	2.18	1.93	2.07†	4.72	3.59	4.10
J. L.	23	36.43	33.33	35.20	17.66	16.33	17.04	2.15	2.00	2.07	4.69	2.88	3.94
Total....	60	37.33	33.33	35.34‡	18.00	16.01	17.09	2.27	1.93	2.07‡	4.72	2.88	3.98
S. D.				0.74			0.44			0.19			0.38

* 19 determinations.
† 16 determinations.
‡ 58 determinations.

TABLE II. PART A

Average analytical values according to caries class for each patient on dentin (percentages based on inorganic weight)

CARIES CLASS	NO. TEETH	Ca PER CENT	P PER CENT	Ca/P PER CENT	CO_2 PER CENT
		Patient E. R.			
0	2	34.91*	17.46	2.03*	3.43
1	10	35.86	17.20	2.09	3.90
2	5	35.18	17.30	2.03	3.92
3	3	35.83	16.81	2.13	4.05
		Patient C. L.			
0	1	35.51	17.44	2.03	4.12
1	7	35.34†	17.05	2.06†	3.91
2	7	35.19	17.03	2.09	4.29
3	2	34.82	16.78	2.07	4.09
		Patient J. L.			
0	3	34.59	17.01	2.03	3.73
1	3	35.27	17.04	2.07	4.13
2	5	34.78	16.94	2.06	4.47
3	12	35.52	17.09	2.08	3.80

* 1 determination.
† 6 determinations.

The Ca, P and CO_2 percentages are graphed (*fig. 1*) for each patient separately in the various caries classes. Six determinations were made of the CO_2 content of enamel. The percentage values (inorganic

weight) are 2.30, 2.47 (caries class 0); 2.66 (caries class 1); 2.74, 2.24 (caries class 2) and 2.52 (caries class 3); average 2.47. Four of the samples were obtained from teeth of patient J. L. and two from patient C. L.

TABLE II. PART B

General average for each caries class for all the patients

0	6	34.80‡	17.23	2.03‡	3.70
1	20	35.60§	17.12	2.08§	3.94
2	17	35.06	17.08	2.06	4.23
3	17	35.49	17.00	2.09	3.88

‡ 5 determinations.
§ 19 determinations.

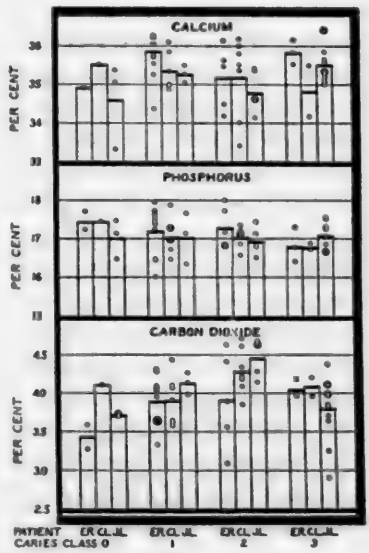

FIG. 1. Ca, P and CO_2 percentages according to caries class and to patient within each class. Averages are represented by horizontal bars and individual values by circles. Note lack of variation in values with increasing severity of caries.

DISCUSSION

The values for Ca, P and CO_2 percentages in the dentins have been grouped according to the caries classes of the teeth and also according to patients, since there was considerable difference in the amount of

caries found in the mouths of the 3 individuals. That is, a plurality of the teeth from patient E. R. fall in class 1, most of the teeth of C. L. are found in classes 1 and 2, and the majority of the teeth of J. L. are in the severe caries class 3. Thus, if there are consistent differences in the inorganic constituents of teeth found in different caries classes, one would expect to find these same differences existing to a lesser degree between the three patients.

However, when the values for the different constituents are plotted against the caries classes by patients (*fig. 1*), apparently no such differences exist. For example, the average values for calcium (Table II) are: class 0, 34.80; class 1, 35.60; class 2, 35.06; and class 3, 35.49. The widest range between the class averages is no greater than the standard deviation of the calcium values. Likewise, for calcium values averaged by patients (Table I, part b), i.e., E. R., 35.64; C. L., 35.22; and J. L., 35.20; the range is less than the standard deviation for the calcium values. Similar constancy is found in the phosphorus values (*fig. 1*). The averages for the caries classes are as follows: class 0, 17.23; class 1, 17.12; class 2, 17.08; and class 3, 17.00. Since the standard deviation for the phosphorus values is 0.44, the greatest difference between caries class averages is much less than the standard deviation. Likewise if the patient averages are considered (Table II, part b). i.e., E. R., 17.19; C. L., 17.03; and J. L., 17.04, the differences are again less than the standard deviation for all the phosphorus values.

This "remarkable constancy" in the composition of the inorganic portion of tooth substance has been noticed before on analysis of whole carious or sound teeth (6, 14). The constancy of both calcium and phosphorus in the inorganic portion of the tooth, however, is even more remarkable when considered in the light of previous findings on these same teeth (15). The values for the dentine of these same teeth have shown increasing moisture and organic and decreasing inorganic percentages with increasing severity of caries. Thus, although the inorganic content of carious dentine is lower than that of non-carious, the calcium and phosphorus percentages remain the same. This would mean apparently that in the process of decalcification, whether it occurs before or after the carious lesion[3] appears in the tooth, the inorganic salt is removed as a unit. This removal of inorganic salts

[3] Carious tissue is removed before analysis.

as units, leaving a tissue of exactly identical calcium and phosphorus content, has long been recognized in the studies of bone metabolism (8, 17). Studies on whole teeth by numerous investigators have indicated that the same type of process occurs in caries. Similar constancy for enamel from sound vs. carious teeth has been reported by Armstrong and Brekhus (1). The studies reported here on pure dentin indicate that in this tissue the inorganic portion seems to have some fundamental chemical unit which is removed in decalcification *in toto*, the remainder being chemically identical with the intact, non-carious tissue.

The CO_2 percentages show more variation than either the calcium or phosphorus (*fig. 1*). When considered according to caries classes (*fig. 1*), the averages range from 3.70 to 4.23 per cent, tending to increase from class 0 to class 2 and to decrease for class 3, although the differences are not statistically significant. Considering the teeth by patients within the various classes, in classes 1 and 2 there is a gradual increase in the values for CO_2 from the patient with the least to the patient with the most carious mouth; and in classes 0 and 3, the same increase is noted between the values of patient E. R. and patient J. L. Constancy of the carbonate content of *whole* carious teeth and non-carious teeth has been noted before (14), but differences in the sound dentin from carious and non-carious teeth have not been shown. Further work will be needed to find whether these tendencies are real.

Most of the enamel samples were not analyzed for CO_2 because of insufficient material; however, the few values that were obtained are given above. The average for the CO_2 analyses on 6 enamel samples, 2.47 per cent, is lower than the average for 60 dentin samples, 3.98 per cent (Table I, part b). Similar differences have been noted by Bowes and Murray (3) who report values of 1.95 per cent for unashed enamel and 3.18 per cent for unashed dentin (basis of dry weight). Armstrong and Brekhus (1) report average CO_2 values of 3.05 per cent and 3.64 per cent for alcohol-ether extracted dried enamel and dentin, respectively.

Considerable confusion exists in the discussion about the chemical changes in teeth accompanying caries. A great deal of this confusion is due to the fact that early workers (4), interested in the chemical effects of caries, analyzed sound dentine and compared it to the decal-

cified carious dentine. Of course, low inorganic percentages were found with proportionate percentage increases in organic matter and water. Later, whole sound teeth were compared with whole carious teeth (6, 11, 12, 14, 22). The varying amounts of enamel and dentin present vitiated these results. More recently, the dentine composition of sound teeth has been compared to the composition of the sound dentin from carious teeth by Murray and Bowes (20). Their report of a loss of calcium, but not of phosphorus, in the sound dentin of carious teeth has not been confirmed by the analyses reported herewith. However, the relatively small number of teeth in each caries class (Table II, part b) and the "remarkable constancy" of the composition of the inorganic portion of tooth substance may be responsible for the lack of confirmation. Fish (7) reported that sound dentin has the same calcium content as noncarious dentin from carious teeth.

To date, these data are the first reported on all the dentin of the teeth studied. This fact may be responsible for the differences noted between the average values for Ca, P and CO_2 of Table II and those of other investigators; all of these average values are tabulated (Table III) for ease of comparison. The data have all been recalculated to the basis of dry weight percentages. The values for calcium and phosphorus reported herewith are similar to those of Armstrong and Brekhus (1) and Logan (18), but lower than those of Murray and Bowes (20). Our CO_2 values are lower than any reported before, perhaps due to the better sampling permitted by the separation method used.

The question as to whether the molecular constitution of the mineral phase of dentin is identical with that of enamel has been argued from several angles. The identity of the x-ray diffraction patterns of enamel and dentin (2) is strong presumptive evidence that the principal components are the same. However, Armstrong and Brekhus hold, from chemical analyses, that the mineral phases are different. The arguments which may be educed from the data presented in this paper are summarized as follows:

1. The average Ca:P ratio (2.07) for dentin is practically the same as has been given for whole teeth, 2.04 (13) and 2.10 (14), and for enamel, 2.03 (1) and 2.15 (20), and for hydroxylapatite, 2.15.

2. The average calcium and phosphorus values for dentin, 35.34

and 17.09, respectively, are nearly the same as those given for enamel, 35.41 and 17.45, respectively, by Armstrong and Brekhus (1).

These statements seem to imply that the molecular states of the calcium phosphates in enamel and dentin are identical. However, the acknowledged differences in the CO_2 percentages for the two tissues may be offered as evidence to the contrary. Our concept of the state of the mineral phase (9) may give an explanation for this difficulty. Thus, if the enamel and dentin mineral phases are coacervated particles of hydroxylapatite with occluded, adsorbed or interstitially crystallized carbonates and other salts, the enamel may differ from the dentin only in the quantities of these latter materials around the particles of hydroxylapatite.

TABLE III

Comparison of analytical values for calcium, phosphorus and carbon dioxide on dentin (percentage dry weight)

INVESTIGATOR	ARMSTRONG	LOGAN*	MURRAY AND BOWES		FRENCH ET AL.†
State of Dentin	Sound	Sound	Sound	Carious	Sound + Carious
Ca	26.18	26.6	27.79	27.98	26.1
P	12.74	12.7	13.81	13.87	12.6
CO_2	3.57	3.17	3.18	3.19	2.94

* Recalculated from Table V, p. 383.

† Recalculated from the average values of Table I, part b, using the average inorganic content (69.3 per cent) from the data on these same teeth given by LeFevre and Manly, Table IV, p. 240.

SUMMARY

1. All the dentin from each of 60 teeth (taken from 3 patients), analyzed for Ca, P and CO_2, gave the following averages plus or minus the standard deviations: Ca—35.34 $\pm$ 0.74, P—17.09 $\pm$ 0.44, and CO_2—3.98 $\pm$ 0.38 (based on inorganic weights).

2. When the values for the teeth of the 4 caries classes were analyzed and compared, it was evident that Ca and P values exhibited a "remarkable constancy," showing no tendency to change with increasing degree of caries. The CO_2 values tended to increase from caries class 0 to class 2, but the differences are not statistically significant.

3. Evidence is brought out to show that the principal inorganic component of dentin is probably hydroxylapatite, the carbonates presumably being adsorbed, occluded or interstitially crystallized.

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SALIVA AND ENAMEL DECALCIFICATION

III. Autolysis

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Autolysis in saliva is dependent on 3 factors: (1) The presence of a complex carbohydrate in the saliva. (2) The presence of an enzyme, or enzymes in saliva capable of hydrolyzing the complex carbohydrates into reducing sugars (diastatic action). (3) The hydrolytic action of the enzyme, or enzymes, on the complex carbohydrate converting it into a fermentable sugar (autolysis).

Carbohydrate content of the saliva—The saliva contains mucin in varying amounts but the factors influencing the salivary concentration of mucin have not been determined. Mucin is a glycoprotein composed of carbohydrate and protein radicals. According to Mathews (3) the carbohydrate radical makes up 36.9 per cent of salivary mucin. The presence of a carbohydrate in saliva has been recorded by Michaels (4), Kirk (5, 6), Simon (7) and others. The carbohydrate in the mucin of freshly secreted saliva will not reduce alkaline copper solutions and is not fermentable.

While quantitative determinations of salivary mucin have not been made by the author, quantitative determinations of the carbohydrate in unstimulated mandibular and parotid saliva have been made on high and low carbohydrate diets by the use of the saliva separator (2). The technique used to determine salivary carbohydrate in terms of mgm. of glucose per 100 cc. of saliva is described in a previous paper (1).

A series of experiments was instituted in an attempt to determine the changes taking place in the saliva with a diet in which the carbohydrates were eliminated as far as was consistent with health and with a diet containing an excess of carbohydrates.

Before and during the first series of experiments all refined sugars and starches and starchy vegetables were eliminated from the diet

and food consumption limited to about 1200 to 1500 calories per diem including 1 glass of milk, 3 or 4 oranges, meat, eggs and green vegetables. Before and during the second series of experiments refined sugars and starches and starchy vegetables were eaten in abundance, daily caloric intake being about 3000 calories of which at least 2000 were from sugars and starches.

For 3 days before starting the low carbohydrate diet a record was kept of the carbohydrate content of unstimulated whole saliva and was continued for 6 days on the diet, saliva being collected before breakfast. At the end of 4 days on the low carbohydrate diet a minimum concentration was reached (*fig. 1*). After the first 3 days

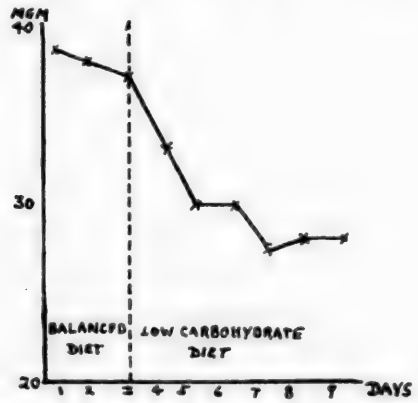
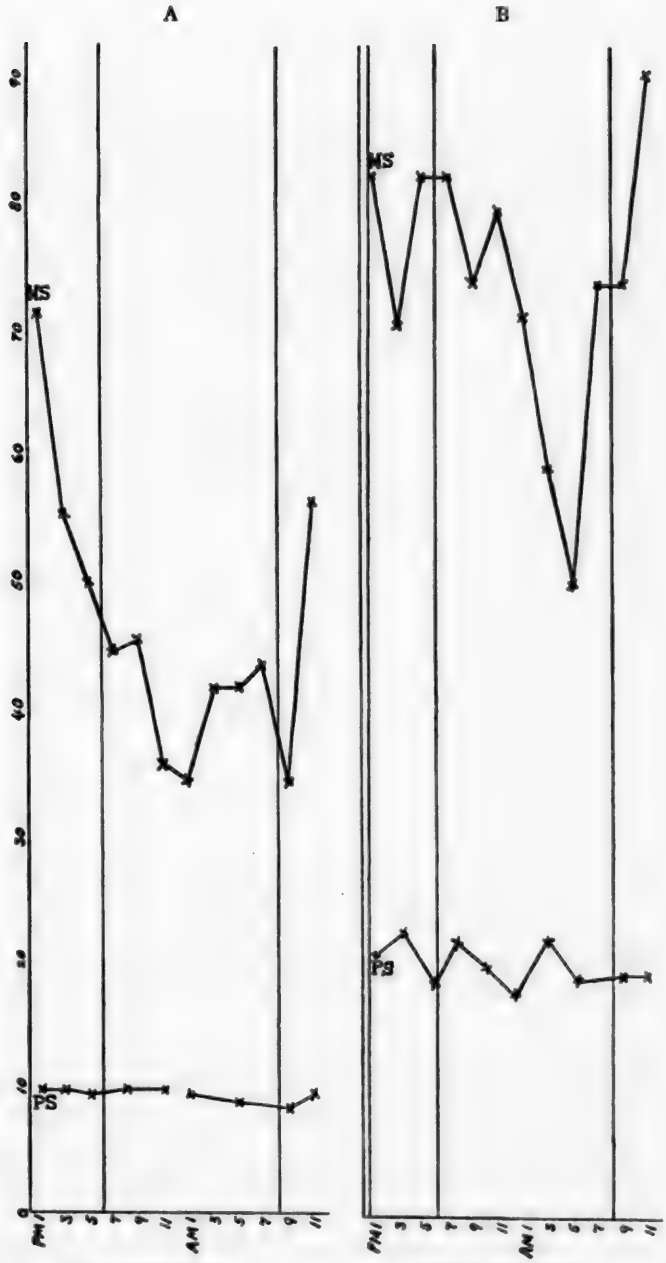


FIG. 1. Carbohydrate content in mgs. of glucose per 100 cc. of saliva

on a balanced diet the carbohydrate content decreased steadily for 4 days on the low carbohydrate diet until a minumum was reached on the seventh day after starting the experiment (fifth day of low carbohydrate diet). A diet as outlined above, low in carbohydrates, was continued for 3 weeks, tests being made every 2 hours over a 24 hour period. Tests included quantities, carbohydrate content, diastatic action and alkaline reserve for both parotid and mandibular salivas. The results of the carbohydrate determinations are shown in *fig. 2A*. The light lines represent the thin, transparent parotid saliva; the heavy lines the thick, opaque mandibular saliva. Meal periods are indicated by vertical lines. The hours at which salivary collections



Low Carbohydrate High Carbohydrate

FIG. 2. Carbohydrate content in terms of mgs. of glucose per 100 cc. saliva. A. Low carbohydrate diet. B. High carbohydrate diet.

were made are plotted on the abscissae; the amounts of carbohydrate in mgms. of glucose per 100 cc. of saliva on the ordinates. On account of insufficient parotid saliva and low concentration it was impossible to make the carbohydrate test every two hours and a few of the tests were split into two 12 hour periods, indicated by a break in the graph lines between 11 P.M. and 1 A.M.

The high carbohydrate diet was started subsequently and continued for 8 days, similar tests being made. The results are plotted in *fig. 2B*.

A comparison of A and B (*fig. 2*) shows that while there is an increase in the carbohydrate content of the parotid saliva on a high carbohydrate diet, the amount on both diets is insignificant. The contrast in the carbohydrate content of the mandibular saliva on the diets is significant. On the low carbohydrate diet a high point was reached about noon after which there was a rapid decline, with mild fluctuations and marked and rapid increase in the late morning hours of the following day. On the high carbohydrate diet the carbohydrate content of the mandibular saliva averaged about twice the amount on the low carbohydrate diet, the low concentration being limited to about 4 hours between 2 A.M. and 6 A.M.

Fig. 3 shows the carbohydrate content of the unstimulated whole saliva on the contrasting diets for the 24 hour period. These charts are plotted on a smaller scale.

While the maximum sugar content of 66 mgs. in whole saliva on high carbohydrate diet is inadequate to produce any marked change in the H-ion concentration of the buffered saliva, tests have shown that about 60 per cent of this sugar will be found in the gelatinous mucin precipitate formed from the whole saliva standing for 1 hour at room temperature.

In a preliminary report (1) a method of separating the mucin precipitate from the stimulated saliva was described, 100 cc. of saliva yielding approximately 1.5 cc. of gelatinous mucin precipitate. A second report (2) shows the amount of parotid and mandibular saliva secreted per hour when stimulated and unstimulated, 100 cc. of stimulated whole saliva containing approximately 25 cc. of mandibular saliva and 100 cc. of unstimulated whole saliva containing 50 cc. of mandibular saliva. On this basis 100 cc. of unstimulated whole

saliva would yield 3 cc. of mucin precipitate. Tests by the author have shown that this 3 cc. of mucin precipitate will contain about 60 per cent of the carbohydrate contained in the original 100 cc. of whole saliva. *Fig. 3B* shows that 100 cc. of unstimulated whole saliva on a high carbohydrate diet contained an average of 55 mgs. of sugar. After precipitation for 1 hour at room temperature 60 per cent of this sugar, or 33 mgs., will be found in the 3 cc. of the gelatinous

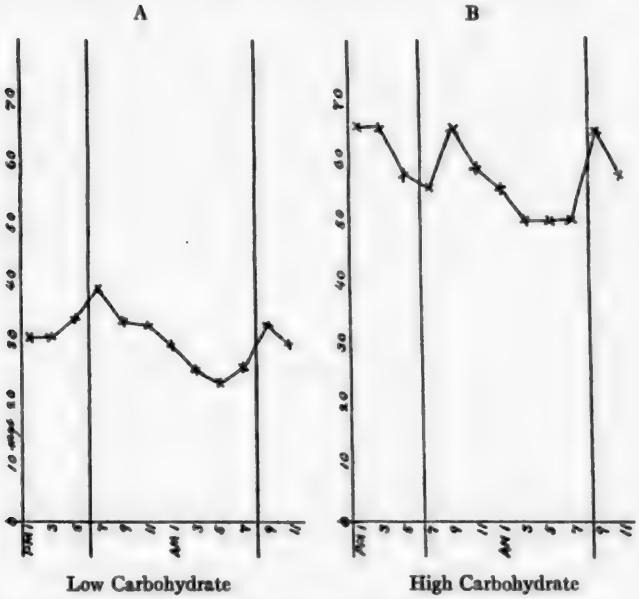


FIG. 3. Carbohydrate content. Whole saliva. A. Low carbohydrate diet. B. High carbohydrate diet.

mucin precipitate, or the mucin precipitate will have a sugar concentration of 1.1 per cent.

Diastatic action of saliva. One of the most important functions of the saliva is its diastatic action, whereby disaccharides and polysaccharides are hydrolyzed into monosaccharides for absorption and utilization by the body as energy and heat. By the use of the saliva separator (2) it became possible to study the diastatic action of the parotid saliva and the mandibular saliva separately and also study

some of the factors influencing the diastatic activity of the saliva from the 2 sets of glands.

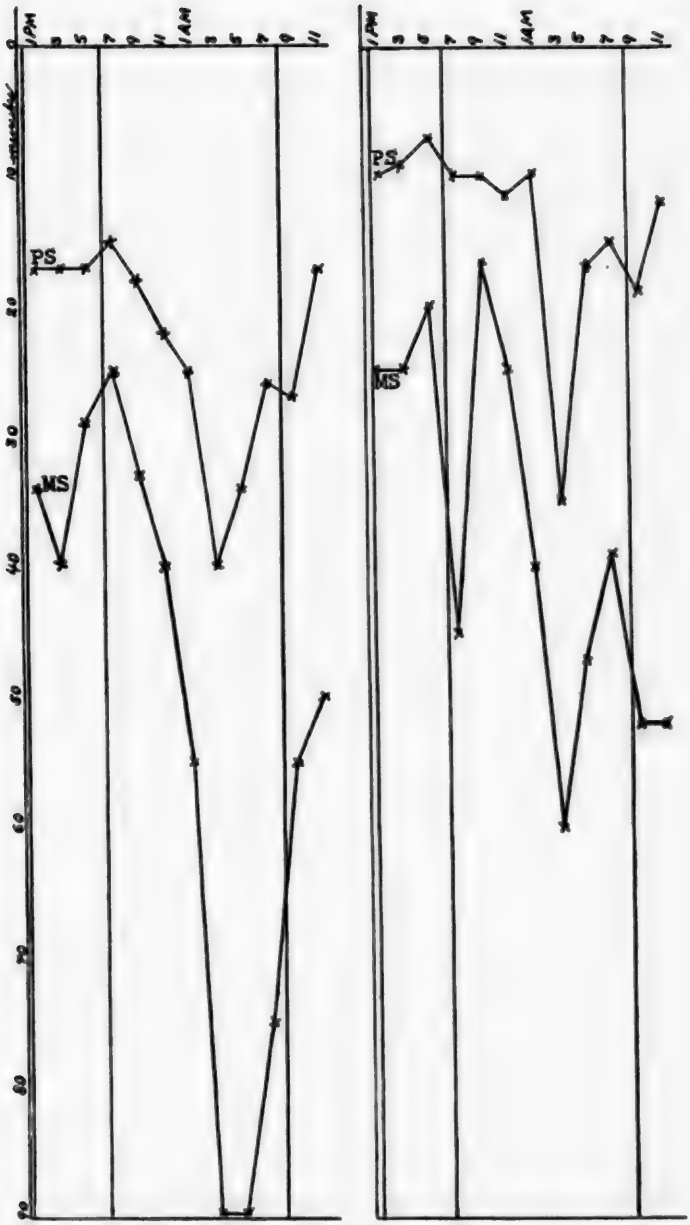
The method adopted for determining the diastatic action was developed by the writer along lines suggested by Mathews (3) as follows:

.2 gram of commercial corn starch (Kingsford's) was dissolved in 100 mls. of distilled water and boiled for fifteen minutes, stirring constantly. When cooled to room temperature the volume was restored to 100 mls. by adding distilled water. Shake starch solution and measure accurately 10 mls. into a test tube and heat to 37°C. Shake the saliva to be tested vigorously for 2 minutes and add 2 mls., of the saliva to the starch solution and place in the incubator at 37°C. Spot with 1 drop of a N/100 Iodine-Potassium Iodide solution at intervals until the end point is reached, using .5 ml. of the starch-saliva solution. The time required to reach the end point in minutes is recorded as the diastatic index. If complete decolorization is taken as the end point it is sometimes very indefinite. The writer found it more satisfactory to establish the end point when the yellow color of the iodine was plainly visible immediately after stirring slowly with a glass rod for 15 seconds, but a pale violet color may then develop as the iodine is decolorized. The secret of uniform results is the thorough shaking of the saliva and the accuracy of the measurements of the ingredients used in the test. The tests should be made immediately after collecting the saliva and neither the starch solution nor the iodine solution should be more than 2 days old.

Diastatic tests were made every 2 hours over a 24 hour period on fresh, unstimulated parotid and mandibular saliva on the high and low carbohydrate diets. *Fig. 4* shows the results of these tests. The vertical lines indicate meal times; the abscissae represent the time at which the collection of saliva for that test was started and the ordinates represent the number of minutes required to reach the end point by spotting with N/100 iodine solution and is recorded as the diastatic index. The lower the index the greater is the diastatic activity. The light lines represent the parotid saliva and the heavy lines represent the mandibular saliva.

Fig. 4 indicates that both the parotid and mandibular saliva on both diets are subject to rapid and marked changes in diastatic activity, both showing a marked decrease in the early morning hours. The diastatic action of the parotid saliva is greater than that of the mandibular saliva at all times. It will also be observed that the diastatic action of both salivas is greater on the high carbohydrate diet and the period of decreased activity at night is of much shorter duration and less marked on this diet, especially with the mandibular saliva.

Twenty-four hour tests were also made on the unstimulated, whole



Low Carbohydrate High Carbohydrate

FIG. 4. Diastatic test with starch. Number of minutes to decolorize with N/100 I sol. A. Low carbohydrate diet. B. High carbohydrate diet.

saliva on the contrasting diets, the results of which are shown in *Fig. 5*.

Fig. 5 shows the same general trends as the separated saliva but more clearly indicates the increased diastatic action on the high carbohydrate diet, and also shows that the diastatic index follows the same general trends as the carbohydrate content of the whole saliva.

Autolysis. The effect of the diet on some of the chemical and physical properties of saliva has been shown. An attempt will now be made to show that the complex carbohydrate in the saliva is hydrolyzed into a fermentable sugar in the stagnant saliva by the ptyalin.

If 10 cc. of saliva, having a maximum diastatic index of 25 and a minimum carbohydrate content of 40 mgs., be saturated with thymol (1) and incubated for 15 hours, then evaporated to 5 cc. and tested for a reducing sugar with 2.5 cc. of Benedict's qualitative solution a positive reaction will be obtained. If 10 cc. of the same specimen of saliva, used as a control, be heated to 75°C. for 10 minutes, then cooled to 37°C. and saturated with thymol and incubated for the same length of time and tested in the same manner, a negative reaction for reducing sugar will be obtained due to the destruction of ptyalin.

Provided there is no sialaporia or sialorrhea and the diet of an individual for the previous 4 or 5 days includes a liberal amount of refined carbohydrates, a reducing sugar will be found in the incubated saliva collected and tested as follows:

> After a heavy carbohydrate meal and a 4 hour rest of the salivary glands any amount of saliva up to 50 cc. or 60 cc. is collected at the rate of about 1 cc. per minute divided into 10 to 15 minute periods; about 3 to 5 cc. of unstimulated saliva are collected in from 7 to 10 minutes; paraffin is then chewed vigorously and the stimulated saliva collected with the unstimulated saliva up to 10 cc., this requiring the balance of the 10 to 15 minute period. This is then repeated until the desired amount of saliva is obtained.

This method, with a favorable diet, usually insures sufficient mandibular saliva containing mucin and sufficient parotid saliva with the required diastatic activity to give a positive reaction for reducing sugar in the incubated saliva if fermentation be prevented. Ten cc. of saliva collected in this manner and saturated with thymol will give

the results described above. Thirty cc. of saliva collected in this manner, saturated with thymol, incubated for 3 to 4 hours, evaporated to 5 cc. and tested as above described will give a positive reaction for reducing sugar. Thirty cc. of the same specimen of saliva heated to 75°C. for 10 minutes, cooled to 37°C., saturated with thymol and incubated for the same length of time and tested in the same manner

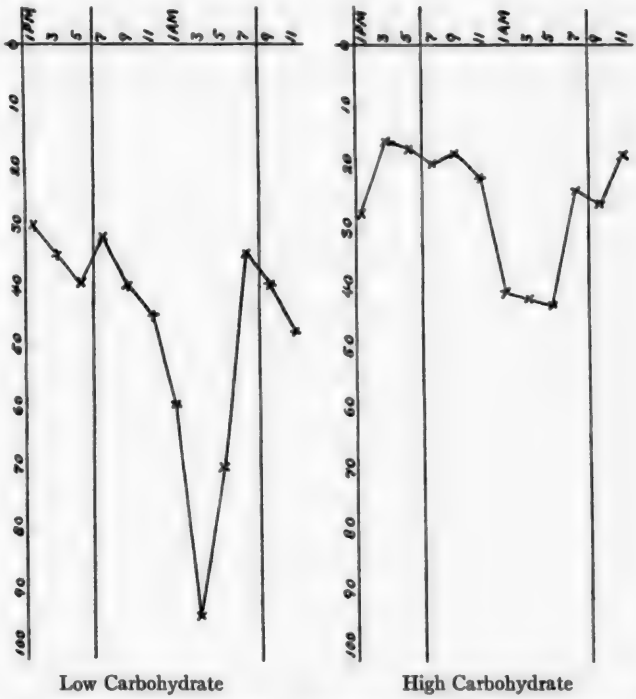


FIG. 5. Diastatic test with starch. Whole saliva. A. Low carbohydrate diet. B. High carbohydrate diet.

will give a negative test for reducing sugar. These tests show a gradual hydrolytic cleavage of the complex carbohydrate molecule and is characteristic of enzyme activity.

Ten cc. of saliva, collected as above described, kept at a temperature of 7°C., which prevents fermentation, for 48 hours and shaken vigorously every few hours during the day, will give a positive sugar reaction

with Benedict's qualitative solution. The same amount of the same specimen of saliva heated to 75°C. for 10 minutes and kept at the same temperature of 7°C. for the same length of time will give a negative result when tested in the same manner.

That the hydrolysis which takes place in the stagnant saliva is not caused by bacterial enzymes is shown by the following experiment:

A quantity of saliva collected as above described was shaken vigorously with an excess of thymol crystals for 2 minutes; dextrose broth was then inoculated with the saliva-thymol mixture every 15 minutes for 3 hours. After incubating for 48 hours dextrose agar was inoculated with .5 cc. of the dextrose broth and placed in the incubator for 72 hours. Examination of the agar cultures at the end of the 72 hours showed that bacteria in the saliva were not destroyed immediately after agitating with thymol but the saliva became sterile within 15 minutes.

Jay (8) showed that there was an increase in the number of *L. acidophilus* when the carbohydrate content of the diet was increased and a decrease when the carbohydrate was decreased,

The saliva contains a complex carbohydrate and amylolytic enzymes. That the complex carbohydrate is hydrolyzed by ptyalin into fermentable sugar is indicated by the following: 1. The physical changes take place in stagnant saliva as a result of the hydrolytic action of ptyalin on the carbohydrate moiety of the mucin. Both occur simultaneously and both cease when the saliva is heated to 75°C. for 10 minutes (destroying the ptyalin (1)). 2. The hydrolysis of the complex carbohydrate into a reducing sugar is not the result of the action of bacteria or of bacterial enzymes. 3. Physical and chemical changes cease when the saliva is heated to 75°C. for 10 minutes. 4. Physical and chemical changes are retarded by low temperatures. 5. Physical and chemical changes are accelerated at a temperature of 45°C. 6. Precipitation of mucin and hydrolysis of the carbohydrate are hastened by the addition of 0.25 to 0.33 per cent lactic acid (1). 7. There is a gradual hydrolytic cleavage of the complex carbohydrate molecule. (3 to 7 are characteristic of ptyalin activity.) 8. Fresh saliva incubated at 37°C. for 72 hours will contain no reducing sugar either before or after hydrolyzing with acid, because the complex carbohydrate was hydrolyzed by the ptyalin into a fermentable sugar which was fermented into lactic acid by the acidogenic bacteria (1). 9. Fresh saliva heated to 75°C. for 10 minutes, then incubated at 37°C. for 72 hours will not contain a

reducing sugar, but after hydrolyzing with HCl it will contain the usual amount of reducing sugar. The carbohydrate had remained intact due to the destruction of ptyalin (1). 10. There is a gradual decrease in the alkaline reserve on incubating the fresh saliva for 25 hours, due to the gradual accumulation of H-ions caused by the hydrolysis and fermentation of the carbohydrate (1). 11. Stopping fermentation by saturation with thymol and incubating at 37°C. allows the formation of a reducing sugar in the stagnant saliva. Heating the saliva to 75°C. for 10 minutes, cooling to 37°C., saturating with thymol and incubating at 37°C. prevents the formation of a reducing sugar. 12. Fresh saliva, collected as above described, kept at a temperature of 7°C. for 48 hours, to prevent fermentation, will give a positive sugar reaction. The same amount of the same specimen of saliva heated to 75°C. for 10 minutes, then kept at a temperature of 7°C. for 48 hours will give a negative reaction for reducing sugar. 13. Incubation of the saliva of the author will not produce an acid reaction due to adequate potential OH-ions. Incubation of the mucin precipitate will produce an acid reaction. If the precipitate is heated to 75°C. for 10 minutes it does not become acid upon incubation (1). 14. Jay (8) has shown that there is a marked increase in the number of *L. acidophilus* in saliva on a high carbohydrate diet and a decrease on a low carbohydrate diet. 15. Michaels stated that, "The saliva of adolescence contains a dextrinic principle (glycogen) susceptible of fermentation under the influence of ptyalin in the presence of earthy salts. In this way is obtained dissolution of the earthy salts by the substitution of lactic acid for carbonic acid" (4).

The above tests were made on the saliva of the author with occasional tests on the saliva of other individuals to check against individual abnormality. The physical condition of the author has been checked semi-annually and reported as excellent.

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A COMPARISON OF THE BACTERIAL FLORA OF DIFFERENT MOUTHS[1]

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Few comparisons have been made of the bacterial flora of different mouths and the extent of the variations occurring from mouth to mouth are not known. The observations of Jeannin (1904) and Brailovsky-Lounkevitch (1915) indicate that changes in the flora take place during early life. These occur with the inception of feeding and again when the eruption of the teeth establishes the gingival crevice enabling the anaerobic fusiform and spirochetal organisms to become established. Pickerill and Champtaloup (1913) found that the mouths of Maori children contained more filamentous and spirochetal organisms than were present in European children. Using cultural methods which give a more limited knowledge of the flora as a whole Kligler (1915) claimed that "leptothrix," "cladothrix" and lactobacilli were more common in mouths with active caries. Thomson and Thomson (1932) observed that organisms prominent in cultures from one mouth were present only in small numbers in others. The indefinite nature of this scanty literature indicates the need of further studies in this field.

MATERIALS AND METHODS

In all, 20 patients are considered in this report. They represent 5 series each of 4 patients having mouths free from gingivitis and caries, with slight caries, with rampant caries, with gingivitis, or with pyorrhoea. Six specimens from each mouth were studied. They consisted of mouth washings, a material collected from the posterior surfaces of last lower molars, from gingival crevices of central incisors, from unfilled fissures, from buccal surfaces of upper molars and from the buccal mucosa. In the rampant caries and pyorrhoea groups suitable cavities or pockets were used as the sources of material instead of fissures and crevices. All instruments and materials used were sterile, and precautions

[1] The study upon which this report is based was made possible by funds granted by the Carnegie Corporation of New York.

were taken to reduce to a minimum contamination from other parts of the mouth. Mouth washings were collected by washing the mouth for 1 minute with 10 cc. of 0.85 per cent sodium chloride solution. To prevent masking of the basic flora from particular areas by superficial contamination the mouth was rinsed twice, as above, and then the chosen areas were dried and isolated by means of absorbent cotton rolls. Samples of material were then taken on appropriate dental instruments. These were shaken with glass beads to make an even suspension and smeared over wide areas on 4 microscope slides. These smears were allowed to dry at room temperature.

Successive smears were stained by Gram's method until a satisfactory preparation was obtained. The remaining slides were stained with the Ljubinsky, iodin and Fontana stains. The percentage occurrence of different morphological groups of organisms was then estimated by direct microscope examination (Bibby, 1938). In these studies particular attention was paid to the percentages of cocci and bacilli. In the Gram-stained smears the organisms were differentiated primarily into filaments, bacilli, and cocci. The filaments and bacilli were each subdivided into long and short types and the bacilli of each group again divided according to whether the ends were round or square. In this grouping fine long fusiform organisms (*Fusiformis polymorphus*) were included with the Gram-negative filaments, while other fusiform types under 15μ in length were placed with the Gram-positive or Gram-negative bacilli. Since the subgrouping of the bacilli proved to have little significance the subgroups will be reported individually only in a few instances. The unsatisfactory state of our knowledge of spirochetal organisms and the difficulty in making differential counts in Fontana-stained smears limited the usefulness of the findings in smears stained by this method. Consequently such counts were made only on about one third of the specimens. The percentage totals of all spiral forms were determined by estimating their incidence in approximately 1000 organisms.

FINDINGS

The inconstancy of the results makes a detailed presentation pointless. However, to demonstrate the nature of the findings the percentage occurrence of the different groups of organisms found in the 20 mouths in one of the 6 areas is presented in Table I. The averages of the percentage occurrences of the various types of organisms in each site for each group of patients are given in Table II. In this table those percentage averages which were calculated from fairly uniform groups are marked.

Considerable differences in the percentage occurrence of various organisms appeared even within the series of 4 smears from the same sites in dentally-similar mouths. Indeed, as may be seen from Table II, in only 23 of 210 such series did the percentages approximate closely. On the whole the variations in percentages of organisms between different locations in the same mouth were more distinct than those existing between identical locations in the different mouth series. In no mouth series did all the percentages, or their averages, of any

group of organisms in the different areas show parallel variations in relation to any other series. This condition was most closely ap-

TABLE I

Percentage occurrence of groups of organisms in material from gingival crevices in different mouths

SOURCE OF SMEARS		PERCENTAGES OF TOTAL ORGANISMS COUNTED*							SPIRALS
		Cocci		Bacilli		Filaments			
Patient	Type	Gram +	Gram −	Gram +	Gram −	Gram +	Gram −	Ljb +	
A	Normal	42.7	36.6	2.2	7.2	2.7	8.3	5.9	0.3
B	"	35.1	18.2	25.5	9.4	0.6	10.7	4.5	0
C	"	27.5	49.1	6.6	2.5	5.0	9.1	0.3	0
D	"	17.8	67.1	0.7	7.1	1.4	5.7	5.3	1
Average	"	30.8	35.3	8.8	6.6	2.4	8.5	4.0	
E	Slight Caries	26.5	54.6	0	2.3	3.8	12.4	3.5	7
F	" "	50.0	0	35.0	12.0	0	3.0	0	1.3
G	" "	32.6	11.5	5.7	30.7	0	19.2	0	
H	" "	41.8	2.3	11.5	16.2	14.7	15.1	0	0.8
Average	" "	37.7	17.1	13.1	15.3	4.6	12.4	0.9	
I	Rampant Caries	31.4	56.8	0	0	0	11.5	8.7	0.6
J	" "	19.7	54.6	3.5	9.3	1.1	11.5	6.9	0
K	" "	26.6	50.6	0	7.3	1.3	13.9	4.2	0
L	" "	28.2	49.4	5.5	4.5	3.5	8.5	7.1	0
Average	" "	26.5	52.3	2.3	5.3	1.5	11.4	6.2	
M	Gingivitis	65.8	14.6	0	4.2	1.2	13.9	0.8	5
N	"	29.9	33.0	7.2	2.4	6.6	20.5	9.1	
O	"	53.3	15.3	0	1.6	13.1	16.4	6.2	
P	"	34.6	38.5	4.6	5.5	5.4	10.9	1.4	4
Average	"	45.9	25.4	3.0	3.4	6.6	15.4	4.4	
Q	Pyorrhoea	36.3	36.3	9.0	5.9	8.4	4.1	5.8	0.4
R	"	36.4	25.8	12.5	10.5	7.8	6.5	4.3	
S	"	16.8	65.7	3.1	6.8	4.9	2.2	3.3	
T	"	33.9	42.2	14.6	4.2	2.0	3.1	3.2	
Average	"	30.9	42.5	9.8	6.9	5.8	4.0	4.1	

* Excluding Spirals.

proximated by the Slight Caries Series in which the percentages of Gram-negative cocci were strikingly low in 5 of 6 sites. On the whole

TABLE II

Average percentage occurrence of groups of organisms in series of smears of material from various sites in different mouths

GROUPS OF 4 PATIENTS	SOURCE OF SMEARS	PERCENTAGES OF TOTAL ORGANISMS COUNTED						
		Cocci		Bacilli		Filaments		
		Gram +	Gram −	Gram +	Gram −	Gram +	Gram −	Lib +
Normal	Mouth Washings	46.6**	31.0*	2.1*	7.4	3.7	9.2	1.1
	Fissures	19.2**	55.4**	0.6	5.2*	3.8	15.5	5.7
	Posterior Molars	25.9*	42.1*	1.6	7.2	6.7**	16.6**	6.7
	Smooth Surfaces	28.7**	43.8**	1.7	8.0	4.1	13.8*	1.8
	Gingival Crevices	30.8*	35.3	8.8	6.6	2.4	8.5*	4.0
	Buccal Mucosa	56.6*	23.4*	1.1	3.9	2.6	12.4*	1.7
Slight Caries	Mouth Washings	44.0*	31.5*	7.4	10.4*	3.7	3.7	1.3
	Fissures	28.9	23.1	9.5	13.7*	9.2	15.5	0**
	Posterior Molars	25.7*	15.4	7.4	18.8	7.2	25.3	0.4
	Smooth Surfaces	30.1*	23.9	8.6	15.3	3.2	18.7	
	Gingival Crevices	37.7*	17.1	13.1	15.3	4.6	12.4	0.9
	Buccal Mucosa	76.8**	8.6	1.9	4.2	5.2	5.6	0*
Rampant Caries	Mouth Washings	49.8*	27.3*	5.7	4.5	1.5	10.8	6.4
	Fissures	25.8	44.8*	5.6	8.4*	5.1	11.2	3.3
	Posterior Molars	27.9	38.7*	0**	1.3*	5.3	20.8*	6.7
	Smooth Surfaces	24.0*	48.2**	1.1	4.1	2.8	19.8**	7.9
	Gingival Crevices	26.5*	52.3**	2.3	5.3	1.5	11.4**	6.2
	Buccal Mucosa	72.3*	12.6	1.2	4.9	1.3	7.5**	0.3
Gingivitis	Mouth Washings	37.9*	36.1	4.3	5.2*	4.6	11.5*	1.8
	Fissures	28.7*	56.8**	0.8	2.8	4.5*	5.6**	1.9
	Posterior Molars	27.8**	44.6	4.8	7.9	2.4	12.6**	5.6*
	Smooth Surfaces	31.8*	41.6*	3.6	5.3	2.6	15.0	7.0
	Gingival Crevices	45.9	25.4	3.0	3.4	6.6	15.4*	4.4
	Buccal Mucosa	57.3*	16.5*	0.9	4.9	6.1	13.6	0.4
Pyorrhoea	Mouth Washings	56.6**	23.3*	5.8	4.0*	4.7*	5.1	5.1
	Fissures	31.0**	42.2**	5.3	6.4**	8.2	6.9	3.1
	Posterior Molars	23.5*	41.7*	10.2	5.7	7.3*	11.3*	9.2*
	Smooth Surfaces	30.9*	40.9*	7.6	6.5	7.9*	6.1**	4.3**
	Gingival Crevices	30.9*	42.5*	9.8	6.9*	5.8	4.0*	4.1*
	Buccal Mucosa	58.5**	17.2*	7.4	4.0*	7.2	5.6*	2.0

* = Series variation smaller than percentage of average.
** = Series variation less than half percentage of average.

the variations in the bacterial flora between the mouth series were never sufficiently definite to reflect themselves in the numbers of organisms in all sites in any of the series.

To determine whether less definite differences occurred, a comparison was made of the same sites in different series of mouths. The difficulty of drawing any conclusions from the available figures is exemplified by the findings for the smears from the gingival crevices (Table I). It can be seen that in most instances the percentage variations within the series groups were so wide that the averages had little significance. Therefore, in making comparisons only the more uniform series were considered and these alone are given in the following textual discussion of the findings.

In the *gingival smears* the irregular occurrence of Gram-positive cocci was obvious in all series of mouth. Similar wide variations occurred in the percentages of Gram-negative cocci although they were consistently high (range 49.4–56.8, av. 52.3 per cent) in the rampant caries series. High percentages of Gram-negative filaments were present in the gingivitis series (range 10.9–20.5, av. 15.4 per cent) and the low percentages in pyorrhoea (range 2.2–6.5, av. 4.0 per cent). In the *mouth washings* the outstanding deviation was found in the Gram-positive cocci in the pyorrhoea series (range 51.2–61.0, av. 56.6 per cent). These figures were almost 7 per cent higher than those of any other series, but on the basis of our experimental error (± 4.2 at the 50 per cent range) this difference has no statistical significance. In *fissure smears* the highest percentages of Gram-positive cocci again occurred in the pyorrhoea series (range 26.9–35.1, av. 31.0 per cent). It is interesting to note that material taken from carious cavities (range 0–12.2, av. 5.6 per cent) failed to show the uniform or high percentage of Gram-positive bacilli which could have been expected if the percentage of lactobacilli had been significantly increased. The average percentage of bacilli with the morphology of lactobacilli was 1.9. The outstanding feature of the counts of material from the *posterior surface of molars* was the high percentages of Gram-negative filaments, of which the highest uniform series was from the normal mouths (range 12.9–20.5, av. 16.6 per cent). Smears from the *smooth surfaces* showed no characteristic variations. High

percentages of Gram-positive cocci and a reduction of all other organisms characterized the smears from the *buccal mucosa*.

The information gained by examination of Ljubinsky-stained smears was no more definite than that obtained from the Gram-stained preparations. The highest percentage of Ljubinsky-reacting filaments (range 5.3–14.5, av. 9.2 per cent) was found in the pyorrhoea series of smears from the posterior surface of the molars. As a rule the Ljubinsky-positive filaments constituted less than half of the total filaments. In a few instances diphtheroid Ljubinsky-reacting bacilli were observed.

The examinations and counts of the iodin-stained smears contributed very little information. Occasionally the stain revealed a few indefinitely reacting organisms, generally cocci, some of which were in chains of large closely approximated elements. These types never represented more than 2 or 3 per cent of the total organisms.

Surprisingly low percentages of spirochetal organisms were found even in mouths where there was definite gingival inflammation. The highest percentage of spirochetes found was 8.9 per cent in the smear from the posterior surface of the last lower molar in a patient from the slight caries group. The total percentages often were under 1 per cent. Most of the spiral organisms were of the *Treponema vincenti* or *Treponema buccale* types.

DISCUSSION

These figures offer the first evidence of the distribution of different groups of organisms in the oral flora in various conditions of the mouth. However, this evidence is not conclusive and fails to establish any variations which can be related to the states of the mouth. Most of the figures only serve to emphasize the variation which occurs in the flora of different mouths or even in the different parts of the same mouth. Indeed, from this small series of observations it appears that the latter variations are the more important and it seems that it will be necessary to establish the normal ranges in the flora for different parts of the mouth before it will be possible to recognize variations associated with pathological conditions.

In spite of the irregularities of the results, a few interesting findings present themselves. An outstanding finding is the demonstration of

the relatively high percentages of Gram-negative cocci. These apparently do not show in cultural studies. In connection with the association of various bacterial types with mouth disease, the relatively low percentages of Gram-positive bacilli of the lactobacilli type is noteworthy. They are never very prominent constituents of the oral flora, and in these observations are no more numerous in the rampant caries than the other series. In the pyorrhoetic mouths there is a slight relative increase in the percentages of Gram-positive cocci and a more definite increase in Gram-positive filaments, changes which could be expected in association with suppuration and calculus formation.

SUMMARY

By a direct counting method estimates of the percentage occurrence of different morphological groups of microörganisms were made in smears from 6 locations in each of 20 mouths comprising 5 groups showing different oral conditions.

The variations in the bacterial flora in different parts of the same mouths and the differences in the percentages of organisms in different mouths of the same groups or series were so great that no significant relationship could be established between the oral flora and the state of oral health.

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